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## PROVISIONAL APPLICATION COVER SHEET

To the Commissioner of Patents and Trademarks

Alexandria, VA 22313

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

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TITLE OF THE INVENTION (280 characters max)

PROTEIN C AND ENDOTHELIAL PROTEIN C RECEPTOR POLYMORPHISMS AS INDICATORS OF PATIENT OUTCOME

**PLEASE ASSOCIATE THIS APPLICATION WITH  
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<input checked="" type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fees	Provisional filing fee amount(s)	\$ <u>160.00</u>
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Respectfully submitted,

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**Protein C and Endothelial Protein C Receptor Polymorphisms  
as Indicators of Patient Outcome**

**FIELD OF THE INVENTION**

- 5 The field of the invention relates to the assessment and/or treatment of patients with an inflammatory condition.

**BACKGROUND OF THE INVENTION**

- Genotype has been shown to play a role in the prediction of patient outcome in  
10 inflammatory and infectious diseases (MCGUIRE W. et al. *Nature* (1994) 371:508-10;  
NADEL S. et al. *Journal of Infectious Diseases* (1996) 174:878-80; MIRA JP. et al. *JAMA*  
(1999) 282:561-8; MAJETSCHAK M. et al. *Ann Surg* (1999) 230:207-14; STUBER F. et  
al. *Crit Care Med* (1996) 24:381-4; STUBER F. et al. *Journal of Inflammation* (1996)  
15 46:42-50; and WEITKAMP JH. et al. *Infection* (2000) 28:92-6). Furthermore, septic and  
non-septic stimuli such as bacterial endotoxin and cardiopulmonary bypass (CPB),  
respectively, activate the coagulation system and trigger a systemic inflammatory response  
syndrome (SIRS). Protein C and endothelial cell protein C receptor (EPCR) both play a  
role in the inflammatory response.
- 20 Protein C, when activated to form activated protein C (APC), plays a major role in three  
biological processes or conditions: coagulation, fibrinolysis and inflammation. Acute  
inflammatory states decrease levels of the free form of protein S, which decreases APC  
function because free protein S is an important co-factor for APC. Sepsis, acute  
inflammation and cytokines decrease thrombomodulin expression on endothelial cells  
25 resulting in decreased APC activity or levels. Septic shock also increases circulating  
levels of thrombomodulin, which is related to increased cleavage of endothelial cell  
thrombomodulin. Another mechanism for decreased APC function in sepsis is that  
endotoxin and cytokines, such as TNF- $\alpha$ , down-regulate endothelial cell protein C receptor  
(EPCR) expression, thereby decreasing activation of protein C to APC. Severe septic  
30 states such as meningococcemia, also result in protein C consumption. Depressed protein  
C levels correlate with purpura, digital infarction and death in meningococcemia.

Protein C is also altered in non-septic patients following cardiopulmonary bypass (CPB). Total protein C, APC and protein S decrease during CPB. Following aortic unclamping (reperfusion at the end of CPB) protein C is further activated so that the proportion of remaining non-activated protein C is greatly decreased. A decrease of protein C during  
5 and after CPB increases the risk of thrombosis, disseminated intravascular coagulation (DIC), organ ischemia and inflammation intra- and post-operatively. Patients who have less activated protein C generally have impaired recovery of cardiac function, consistent with the idea that lower levels of protein C increase the risk of microvascular thrombosis and myocardial ischemia. Aprotinin is a competitive inhibitor of APC, and is sometimes  
10 used in cardiac surgery and CPB. Aprotinin has been implicated as a cause of post-operative thrombotic complications after deep hypothermic circulatory arrest.

Septic and non-septic stimuli such as bacterial endotoxin and cardiopulmonary bypass (CPB), activate the coagulation system and trigger a systemic inflammatory response  
15 syndrome (SIRS). A decrease in protein C levels have been shown in patients with septic shock (GRIFFIN JH. *et al.* (1982) *Blood* 60:261-264; TAYLOR FB. *et al.* (1987) *J. Clin. Invest.* 79:918-925; HESSELVIK JF. *et al.* (1991) *Thromb. Haemost.* 65:126-129; FIJNVANDRAAT K. *et al.* (1995) *Thromb. Haemost.* 73(1):15-20), with severe infection (HESSELVIK JF. *et al.* (1991) *Thromb. Haemost.* 65:126-129) and after major surgery  
20 (BLAMEY SL. *et al.* (1985) *Thromb. Haemost.* 54:622-625). It has been suggested that this decrease is caused by a decrease in protein C transcription (SPEK CA. *et al.* *J. Biological Chemistry* (1995) 270(41):24216-21 at 24221). It has also been demonstrated that endothelial pathways required for protein C activation are impaired in severe meningococcal sepsis (FAUST SN. *et al.* *New Eng. J. Med.* (2001) 345:408-416). Low  
25 protein C levels in sepsis patients are related to poor prognosis (YAN SB. and DHAINAUT J-F. *Critical Care Medicine* (2001) 29(7):S69-S74; FISHER CJ. and YAN SB. *Critical Care Medicine* (2000) 28(9 Suppl):S49-S56; VERVLOET MG. *et al.* *Semin Thromb Hemost.* (1998) 24(1):33-44; LORENTE JA. *et al.* *Chest* (1993) 103(5):1536-42). Recombinant human activated protein C reduces mortality in patients having severe sepsis  
30 or septic shock (BERNARD GR. *et al.* *New Eng. J. Med.* (2001) 344:699-709). Thus protein C appears to play an important beneficial role in the systemic inflammatory response syndrome.

- The human protein C gene maps to chromosome 2q13-q14 and extends over 11kb. A representative *Homo sapiens* protein C gene sequence is listed in GenBank under accession number AF378903. Three single nucleotide polymorphisms (SNPs) have been identified in the 5' untranslated promoter region of the protein C gene and are
- 5 characterized as -1654 C/T, -1641 A/G and -1476 A/T (according to the numbering scheme of FOSTER DC. *et al.* Proc Natl Acad Sci U S A (1985) 82(14):4673-4677), or as -153C/T, -140A/G and +26A/T respectively by (MILLAR DS. *et al.* Hum. Genet. (2000) 106:646-653 at 651).
- 10 The genotype homozygous for -1654 C/ -1641 G/ -1476 T has been associated with reduced rates of transcription of the protein C gene as compared to the -1654 T/ -1641 A/ -1476 A homozygous genotype (SCOPES D. *et al.* Blood Coagul. Fibrinolysis (1995) 6(4):317-321). Patients homozygous for the -1654 C/ -1641 G/ -1476 T genotype show a decrease of 22% in plasma protein C levels and protein C activity levels as compared to
- 15 patients homozygous for the -1654 T/ -1641 A/ -1476 A genotype (SPEK CA. *et al.* Arteriosclerosis, Thrombosis, and Vascular Biology (1995) 15:214-218). The -1654 C/ -1641 G haplotype has been associated with lower protein C concentrations in both homozygotes and heterozygotes as compared to -1654 T/ -1641 A (AIACH M. *et al.* Arterioscler Thromb Vasc Biol. (1999) 19(6):1573-1576).
- 20 The human endothelial protein C receptor (EPCR) gene is located on chromosome 20 and maps to chromosome 20q11.2. A representative human EPCR gene sequence with promoter is listed in GenBank under accession number AF106202 (8167 bp). A number of polymorphisms have been observed in the EPCR gene (BIGUZZI E. *et al.* *Thromb Haemost* (2002) 87:1085-6 and FRANCHI F. *et al.* *Br J Haematol* (2001) 114:641-6).
- 25 Furthermore, polymorphisms of EPCR are also described in (BIGUZZI E. *et al.* *Thromb Haemost* (2001) 86:945-8; GALLIGAN L. *et al.* *Thromb Haemost* (2002) 88:163-5; ZECCHINA G. *et al.* *Br J Haematol* (2002) 119:881-2; FRENCH JK. *et al.* *Am Heart J* (2003) 145:118-24; and VON DEPKA M. *et al.* *Thromb Haemost* (2001) 86:1360-2; and
- 30 SAPOSNIK B. *et al.* *Blood* (2004 Feb 15) 103(4):1311-8.).

## SUMMARY OF THE INVENTION

This invention is based in part on the surprising discovery that the combination of predictive SNPs from the Protein C and EPCR can be more accurate predictors of patient outcome than SNPs from either Protein C or EPCR alone.

5

This invention is also based in part on the surprising discovery of protein C SNPs previously uncharacterized in the scientific literature with regards to an association with improved prognosis or patient outcome, in patients with an inflammatory condition. Furthermore, various protein C polymorphisms are provided which are useful for patient screening, as an indication of patient outcome, or for prognosis for recovery from an inflammatory condition.

10

This invention is also based in part on the surprising discovery that EPCR SNPs previously uncharacterized in the scientific literature with regards to an association with improved prognosis or patient outcome, in patients with an inflammatory condition. Furthermore, various EPCR polymorphisms are provided which are useful for patient screening, as an indication of patient outcome, or for prognosis for recovery from an inflammatory condition.

15

This invention is also based in part on the identification the particular nucleotide at the site of a given SNP which is associated with a decreased likelihood of recovery from an inflammatory condition (i.e. 'risk genotype') or an increased likelihood of recovery from an inflammatory condition (i.e. 'protective genotype').

20

This invention is also based in part on the surprising discovery that both EPCR and protein C SNPs alone or in combination are useful in predicting the response a patient with an inflammatory condition will have to activated protein C treatment or treatment with another anti-inflammatory agent or anti-coagulant agent. Whereby the patients having a risk genotype are more likely to benefit from and have an improved response to protein C treatment or treatment with another anti-inflammatory agent or anti-coagulant agent and patients having a protective genotype are less likely to benefit from the same treatment.

25  
30

In accordance with one aspect of the invention, methods are provided for obtaining a

prognosis or predicting ability to recover for a patient having or at risk of developing an inflammatory condition, the method comprising determining a genotype including one or more polymorphism sites in the protein C gene and/or EPCR gene for the patient, wherein said genotype is indicative of an ability of the patient to recover from an inflammatory condition. The method may further involve determination of the genotype for one or more polymorphism sites in the protein C gene and one or more polymorphism sites in the EPCR gene for the patient. The genotypes of the protein C gene and/or EPCR gene may be taken alone or in combination.

The protein C polymorphism site may correspond to position 4732 of SEQ ID NO.: 1 or a polymorphism site linked thereto. Alternatively, the polymorphism site may correspond to position 4732, 4813, 6379, 6762, 7779, 8058, 8915 or 12228 of SEQ ID NO: 1. Using an alternative numbering system, position 4732 corresponds to position 673 according to Foster *et al.* (*supra*).

The protein C polymorphism site may correspond to position 2418 of SEQ ID NO.: 1 or a polymorphism site linked thereto. Alternatively, the polymorphism site may correspond to position 1386, 2583, 3920 or 2418 of SEQ ID NO: 1. Furthermore, the polymorphism site in linkage disequilibrium with position 2418 may correspond to a combination of two or more Protein C polymorphism sites selected from the following: 5867 and 2405; 5867 and 4919; 5867 and 4956; 5867 and 6187; 5867 and 9534; 5867 and 12109; 4800 and 2405; 4800 and 4919; 4800 and 4956; 4800 and 6187; 4800 and 9534; 4800 and 12109; 9198 and 6379 and 2405; 9198 and 6379 and 4919; 9198 and 6379 and 4956; 9198 and 6379 and 6187; 9198 and 6379 and 9534; and 9198 and 6379 and 12109.

The protein C SNPs may also be in linkage disequilibrium with SNPs found both 5' (rs908787) and 3' (rs777566, rs334135, rs777569, rs334142, rs334160, rs334159, rs334151, rs334146, rs777556, rs334144) to the protein C gene (SEQ ID NO:1). These SNPs found 5' and 3' to the protein C gene may be genotyped as an alternative to genotyping protein C SNP 4732 or other protein C SNPs within SEQ ID NO:1 as an indicator of improved prognosis or patient outcome, in patients with an inflammatory condition or assessing a patients risk genotype as described herein.

Genotype may also be determined at a combination of two or more protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO:1 consisting of:

5                   9198 and 5867;  
                  9198 and 4800;  
                  3220 and 5867; and  
                  3220 and 4800.

10           In accordance with another aspect of the invention, methods are provided for further comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

15           The protein C genotype of the patient may be indicative of a decreased likelihood of recovery from an inflammatory condition or indicative of a prognosis of severe cardiovascular or respiratory dysfunction in critically ill patients (risk alleles or risk genotype). Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

20                   4732 C;  
                  4813 A;  
                  6379 G;  
                  6762 A;  
                  7779 C;  
                  8058 T;  
25                   8915 T;  
                  12228 T;  
                  9198 C and 5867 A;  
                  9198 C and 4800 G;  
                  3220 A and 5867 A; and  
30                   3220 A and 4800 G

or

35                   1386 T;  
                  2418 A;  
                  2583 A;  
                  3920 T;  
                  5867 A and 2405 T;  
                  5867 A and 4919 A;  
                  5867 A and 4956 T;  
40                   5867 A and 6187 C;  
                  5867 A and 9534 T;

5 5867 A and 12109 T;  
 4800 G and 2405 T;  
 4800 G and 4919 A;  
 4800 G and 4956 T;  
 4800 G and 6187 C;  
 4800 G and 9534 T;  
 4800 G and 12109 T;  
 9198 A and 6379 G and 2405 T;  
 9198 A and 6379 G and 4919 A;  
 10 9198 A and 6379 G and 4956 T;  
 9198 A and 6379 G and 6187 C;  
 9198 A and 6379 G and 9534 T; and  
 9198 A and 6379 G and 12109 T..

15 The protein C genotype of the patient may be indicative of an increased likelihood of recovery from an inflammatory condition or indicative of a prognosis of less severe cardiovascular or respiratory dysfunction (protective alleles or non-risk genotypes) in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites and combined polymorphism sites consisting of:

20 4732 T;  
 4813 G;  
 6379 A;  
 6762 G;  
 7779 -;  
 25 8058 C;  
 8915 G;  
 12228 C;  
 9198 A and 5867 G;  
 9198 A and 4800 C;  
 30 3220 G and 5867 G; and  
 3220 G and 4800 C.

The EPCR polymorphism site may correspond to position 4054 of SEQ ID NO.: 2 or a polymorphism site linked thereto. Alternatively, the polymorphism site corresponds to  
 35 position 6196, 5515, 4946, 4054, 3402, 3063 or 2973 of SEQ ID NO: 2.

The EPCR SNPs may also be in linkage disequilibrium with SNPs found both 5' (rs 2295887, rs1535466, rs033797, rs1033798, rs1033799, rs2295888, rs666210, rs1415771, rs945959) and 3' (rs1051056, rs632688, rs633198, rs663550) to the EPCR gene (SEQ ID  
 40 NO:2). These SNPs found 5' and 3' to the EPCR gene may genotyped as an alternative to

genotyping EPCR SNPs 4054 or 6196 or other EPCR SNPs within SEQ ID NO:2 as an indicator of improved prognosis or patient outcome, in patients with an inflammatory condition or assessing a patients risk genotype as described herein.

5 In accordance with another aspect of the invention, methods are provided for further comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

10 The EPCR genotype of the patient may be indicative of a decreased likelihood of recovery from an inflammatory condition or indicative of a prognosis of severe cardiovascular or respiratory dysfunction in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites consisting of:

15 6196 G;  
5515 T;  
4946 T;  
4054 T;  
3402 G;  
20 3063 G; and  
2973 C.

The EPCR genotype of the patient may be indicative of an increased likelihood of recovery from an inflammatory condition or indicative of a prognosis of less severe  
25 cardiovascular or respiratory dysfunction in critically ill patients. Furthermore, such a genotype may be selected from the group of single polymorphism sites consisting of:

30 6196 C;  
5515 C;  
4946 C;  
4054 C;  
3402 C;  
3063 A; and  
2973 T.

35 In accordance with another aspect of the invention, methods are provided for identifying a polymorphism in a protein C and/or EPCR gene sequence that correlates with patient prognosis. Where the method comprises obtaining protein C and/or EPCR gene sequence

information from a group of patients, identifying a site of at least one polymorphism in the protein C and/or EPCR gene, determining genotype(s) of the site or sites for individual patients in the group, determining an ability of individual patients in the group to recover from the inflammatory condition and/or correlating genotypes determined with patient abilities and/or potential therapies.

The correlation procedure may be repeated on a patient population of sufficient size to achieve a statistically significant correlation.

The methods may further comprise steps of obtaining protein C and/or EPCR gene sequence of the patient or obtaining a nucleic acid sample from the patient. The determining of genotype may be performed on a nucleic acid sample from the patient.

Where the genotype of the patient corresponding to the nucleotide in position 4732 of SEQ ID NO: 1, is cytosine (C), the prognosis may be indicative of a decreased likelihood of recovery from an inflammatory condition or of severe cardiovascular or respiratory dysfunction in critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4732 of SEQ ID NO: 1, is thymine (T) the prognosis may be indicative of a increased likelihood of recovery from an inflammatory condition or of less severe cardiovascular or respiratory dysfunction in critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4054 of SEQ ID NO: 2, is T, the prognosis may be indicative of a decreased likelihood of recovery from an inflammatory condition or of severe cardiovascular or respiratory dysfunction in critically ill patients.

Where the genotype of the patient corresponding to the nucleotide in position 4054 of SEQ ID NO: 2, is cytosine (C), the prognosis may be indicative of a increased likelihood of recovery from an inflammatory condition or of less severe cardiovascular or respiratory dysfunction in critically ill patients.

In accordance with another aspect of the invention, methods are provided for combining the protein C and EPCR polymorphism site genotype information to improve the predictive value for determining a patient's ability to recover from an inflammatory condition over using either a protein C or an EPCR SNP alone.

5

Group 1 patients have no copies of the EPCR risk allele (4054T) and no copies of the protein C risk allele (4732 C), group 2 patients have at least one copy of the EPCR risk allele (4054T) and at least one copy of the protein C risk allele (4732C). Group 3 patients can have either at least one copy of the EPCR risk allele (4054T) and no copies of the protein C risk allele (4732C) or they can have no copies of the EPCR risk allele (4054 T) and at least one copy of the protein C risk allele (4732C). Group 1 patients are expected to have the best outcomes, group 2 patients are expected to have the worst outcomes and group 3 patients are expected to have intermediate outcomes, with regards to recovering from an inflammatory condition.

15

The inflammatory condition may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumonitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis,

25

emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical  
5 and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglottitis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystis carinii,  
10 pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic  
15 pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

20 The determining of a genotype may be accomplished by any technique known in the art, including but not limited to one or more of: restriction fragment length analysis; sequencing; hybridization; oligonucleotide ligation assay; ligation rolling circle amplification; 5' nuclease assay; polymerase proofreading methods; allele specific PCR; matrix assisted laser desorption ionization time of flight MALDI-TOF mass spectroscopy  
25 micro-sequencing assay; gene chip hybridization assays; and reading sequence data.

In accordance with another aspect of the invention, there is provided a kit for determining a genotype at a defined nucleotide position within a polymorphism site in a protein C gene and/or EPCR gene sequence from a patient to provide a prognosis of the patient's ability to recover from an inflammatory condition, the kit comprising, a restriction enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a labeled oligonucleotide having sufficient complementary to the polymorphism site and capable of distinguishing said alternate nucleotides. The kit may also include one or more of the following: a package; instructions for using the kit to determine genotype; reagents such as buffers, nucleotides and enzymes. A kit as described herein may contain any combination of the following: a restriction enzyme capable of distinguishing alternate nucleotides at a protein C and/or EPCR polymorphism site; and/or a labeled oligonucleotide having sufficient complementary to the protein C and/or EPCR polymorphism site and capable of distinguishing said alternate nucleotides; and/or an oligonucleotide or a set of oligonucleotides suitable for amplifying a region including the protein C and/or EPCR polymorphism site. The kit may also include one or more of the following: a package; instructions for using the kit to determine genotype; reagents such as buffers, nucleotides and enzymes; and/or containers.

The alternate nucleotides may correspond to position 4732 of SEQ ID NO: 1, position 8 of SEQ ID NO: 3 or to a polymorphism linked thereto. The alternate nucleotides may also correspond to one or more of positions 4732, 4813 or 6379 of SEQ ID NO: 1.

The alternate nucleotides may correspond to position 6196 of SEQ ID NO: 3, position 8 of SEQ ID NO: 4 or to a polymorphism linked thereto. The alternate nucleotides may also

correspond to one or more of positions 6196, 5515, 4946, 4054, 3063 or 2973 of SEQ ID  
NO: 2.

The kit comprising a restriction enzyme may also comprise an oligonucleotide or a set of  
5 oligonucleotides suitable to amplify a region surrounding the polymorphism site, a  
polymerization agent and instructions for using the kit to determine genotype.

In accordance with another aspect of the invention, there is provided a kit for determining  
a genotype at a defined nucleotide position within a polymorphism site in a protein C  
10 and/or EPCR gene sequence from a patient to provide a prognosis of the patient's ability  
to recover from an inflammatory condition, the kit comprising, in a package a restriction  
enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a  
labeled oligonucleotide having sufficient complementary to the polymorphism site and  
capable of distinguishing said alternate nucleotides.

15

In accordance with another aspect of the invention, oligonucleotides are provided that may  
be used in the identification of protein C and/or EPCR polymorphisms in accordance with  
the methods described herein, the oligonucleotides are characterized in that the  
oligonucleotides hybridize under normal hybridization conditions with a region of one of  
20 sequences identified by SEQ ID NO:1, SEQ ID NO:2, etc. or their complements.

In accordance with another aspect of the invention, an oligonucleotide primer is provided  
comprising a portion of SEQ ID NO:1, SEQ ID NO:2 or their complements, wherein said  
primer is twelve to fifty-four nucleotides in length and wherein the primer specifically  
25 hybridizes to a region of SEQ ID NO:1, SEQ ID NO:2 or their complements and is

capable of identifying protein C and/or EPCR gene polymorphisms described herein.

Alternatively, the primers may be between sixteen to twenty-four nucleotides in length.

In accordance with another aspect of the invention, methods are provided for patient

5 screening, comprising the steps of (a) obtaining protein C and/or EPCR gene sequence information from a patient, and (b) determining the identity of one or more polymorphisms in the sequence, wherein the one or more polymorphisms may be indicative of the ability of a patient to recover from an inflammatory condition.

10 In accordance with another aspect of the invention methods are provided for patient screening whereby the method includes the steps of (a) selecting a patient based on risk of developing an inflammatory condition or having an inflammatory condition, (b) obtaining protein C and/or EPCR gene sequence information from the patient and (c) detecting the identity of one or more polymorphisms in the protein C gene and/or EPCR gene, wherein  
15 the polymorphism is indicative of the ability of a patient to recover from an inflammatory condition.

In accordance with another aspect of the invention, methods are provided for selecting a group of patients to determine the efficacy of a candidate drug known or suspected of  
20 being useful for the treatment of an inflammatory condition, the method including determining a genotype for one or more polymorphism sites in the protein C gene and/or EPCR gene for each patient, wherein said genotype is indicative of the patient's ability to recover from the inflammatory condition and sorting patients based on their genotype. The method may also include administering the candidate drug to the patients or a subset  
25 of patients and determining each patient's ability to recover from the inflammatory condition. The method may also include the additional step of comparing patient response to the candidate drug based on genotype of the patient. Response to the candidate drug

may be decided by determining each patient's ability to recover from the inflammatory condition.

5 In accordance with another aspect of the invention, methods are provided for treatment of an inflammatory condition in an eligible patient by administering a treatment option, such as a therapeutic agent, after first determining if a patient is an eligible patient on the basis of the genetic sequence information or genotype information disclosed herein. Where the method of treatment of an inflammatory condition in an eligible patient may comprise the following: a) determining if a patient is an eligible patient on the basis of the presence or  
10 absence of one or more risk genotypes in the protein C sequence and/or EPCR sequence; and b) administering a therapeutic agent to the eligible patient. More specifically, the method of treatment of an inflammatory condition in an eligible patient may comprise: a) determining if a patient is an eligible patient on the basis of the presence or absence of one or more risk genotypes in the protein C sequence and/or EPCR sequence; and b)  
15 administering a therapeutic agent selected from among activated protein C (e.g. XIGRIS™ - drotrecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. TIFACOGIN™ - alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ - afelimomab (Abbott)), or other anti-  
20 inflammatory therapeutic agent, to the eligible patient. Furthermore, the therapeutic agent may be activated protein C and/or a derivative thereof (including glycosylation mutants), alone or in combination or in combination with other therapeutic agents as described herein. An improved response to a therapeutic agent may include an improvement subsequent to administration of the therapeutic agent, whereby the patient has an increased  
25 likelihood of survival, reduced likelihood of organ damage or organ dysfunction (Brussels score), an improved APACHE II score, days alive and free of pressors, inotropes, and reduced systemic dysfunction (cardiovascular, respiratory, ventilation, CNS, coagulation [INR> 1.5], renal and/or hepatic).

30 In accordance with another aspect of the invention, methods are provided for treatment of an inflammatory condition in an eligible patient comprising administering a therapeutic agent to an eligible patient. The eligible patient may be a patient having one or more of

the polymorphisms in protein C and/or EPCR that are associated with decreased likelihood of recovery from an inflammatory condition, as disclosed herein or as later discovered. Treatment options, may include: activated protein C (e.g. XIGRIS™ drotrecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. 5 TIFACOGIN™ alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ afelimomab (Abbott)), soluble tumor necrosis factor receptor-immuoglobulin G1 (Roche), procysteine, elastase inhibitor, human recombinant interleukin 1 receptor antagonist (IL-1 RA), and antibodies, inhibitors and antagonists to: 10 an endotoxin (i.e. lipopolysaccharide, LPS, lipotechoic acid and the like, e.g. E-5531 (Eisai)), tumour necrosis factor receptor, IL-6, high-mobility group box 1 (HMGB-1 or HMG-1), tissue plasminogen activator, bradykinin, CD-14, and/or IL-10. Those skilled in the art are familiar with the dosage and administration of these and other treatment options. To determine a patient's eligibility, the presence or absence of polymorphisms in 15 the protein C sequence and/or EPCR sequence, may be determined as described herein.

Activated protein C (e.g. XIGRIS™ drotrecogin alfa-recombinant human activated protein C (Eli Lilly)), tissue factor pathway inhibitors (e.g. TIFACOGIN™ alpha (Chiron) and the like), platelet activating factor hydrolase (e.g. PAFase™ (ICOS) and other PAF-AH 20 enzyme analogues), antibody to tumor necrosis factor- alpha (e.g. SEGARD™ afelimomab (Abbott)), or other anti-inflammatory therapeutic agent, may be useful in the manufacture of a medicament for the therapeutic treatment of an inflammatory condition in a patient having one or more of the polymorphisms in protein C and/or EPCR that are associated with decreased likelihood of recovery from an inflammatory condition. 25 Furthermore these therapeutic agents may be useful in the preparation of an anti-sepsis agent in ready-to-use drug form for treating or preventing sepsis in a patient having one or more of the polymorphisms in protein C and/or EPCR that are associated with decreased likelihood of recovery from an inflammatory condition. 30 In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient in need thereof, the method including administering to the patient an anti-inflammatory agent or an anti-coagulant agent, wherein said patient has

a protein C gene or EPCR gene risk genotype.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient in need thereof, the method including selecting a  
5 patient having a risk genotype in their protein C gene or EPCR gene and administering to the patient an anti-inflammatory agent or an anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a mammal in need thereof, the method including administering  
10 to the mammal an anti-inflammatory agent or an anti-coagulant agent, wherein the mammal has a protein C gene or EPCR gene risk genotype.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a mammal in need thereof, the method including selecting a  
15 mammal having a risk genotype in their protein C gene or EPCR gene and administering to the mammal an anti-inflammatory agent or an anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or  
20 an anti-coagulant agent, including the step of identifying a subject having one or more risk genotypes in their protein C gene or EPCR gene, wherein the identification of a subject with one or more risk genotypes is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

25

In accordance with another aspect of the invention, methods are provided for treating a subject with an inflammatory condition by administering an anti-inflammatory agent or an anti-coagulant agent, the method including administering the anti-inflammatory agent or the anti-coagulant agent to subjects that have a risk genotype in their protein C gene or  
30 EPCR gene, wherein the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for identifying a subject with increased responsiveness to treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, including the step of screening a population of subjects to identify those subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with a risk genotype in their protein C gene or EPCR gene is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, including the step of identifying a subject having a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient, the method including administering an anti-inflammatory agent or an anti-coagulant agent to the patient, wherein said patient has a risk genotype in their protein C gene or EPCR gene.

In accordance with another aspect of the invention, methods are provided for treating an inflammatory condition in a patient, the method including identifying a patient having a risk genotype in their protein C gene or EPCR gene and administering an anti-inflammatory agent or an anti-coagulant agent to the patient.

In accordance with another aspect of the invention, the use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament are provided for the treatment of an inflammatory condition, wherein the patients treated have a risk genotype in their protein C gene or EPCR gene.

In accordance with another aspect of the invention, methods may further comprise determining the patients APACHE II score as an assessment of patient risk and/or determining the number of organ system failures for the patient as an assessment of patient risk. A patient having an APACHE II score when  $\geq 25$  and/or having 2 or more organ system failures may be indicative of increased risk.

Risk genotypes may be selected alone or in combination from the following protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1:

10	4732 C; 4813 A; 6379 G; 6762 A; 7779 C;
15	8058 T; 8915 T; 12228 T; 9198 C and 5867 A; 9198 C and 4800 G;
20	3220 A and 5867 A; and 3220 A and 4800 G
	or
25	1386 T; 2418 A; 2583 A; 3920 T;
30	5867 A and 2405 T; 5867 A and 4919 A; 5867 A and 4956 T; 5867 A and 6187 C; 5867 A and 9534 T; 5867 A and 12109 T;
35	4800 G and 2405 T; 4800 G and 4919 A; 4800 G and 4956 T; 4800 G and 6187 C; 4800 G and 9534 T;
40	4800 G and 12109 T; 9198 A and 6379 G and 2405 T; 9198 A and 6379 G and 4919 A;

9198 A and 6379 G and 4956 T;  
9198 A and 6379 G and 6187 C;  
9198 A and 6379 G and 9534 T; and  
9198 A and 6379 G and 12109 T.

5

Risk genotypes may be selected alone or in combination from the following EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

10

6196 G;  
5515 T;  
4946 T;  
4054 T;  
3402 G;  
3063 G; and  
2973 C.

15

Risk genotype may be an indication of an increased risk of not recovering from an inflammatory condition and may also indicative of an improved likelihood of responding favourable to an anti-inflammatory agent or the anti-coagulant agent. An anti-inflammatory agent or the anti-coagulant agent may be selected from the following  
20 activated protein C; tissue factor pathway inhibitors; platelet activating factor hydrolase; PAF-AH enzyme analogues; antibody to tumor necrosis factor alpha; soluble tumor necrosis factor receptor-immunoglobulin G1; procysteine; elastase inhibitor; human recombinant interleukin 1 receptor antagonists; and antibodies, inhibitors and antagonists to  
25 endotoxin, tumour necrosis factor receptor, interleukin-6, high mobility group box, tissue plasminogen activator, bradykinin, CD-14 and interleukin-10. An anti-inflammatory agent or the anti-coagulant agent is preferably an activated protein C (such as drotrecogin alfa activated or XIGRIS™) or derivative or analog thereof.

Non-risk genotypes or protective genotypes may be selected alone or in combination from

the following protein C single polymorphism sites and combined polymorphism sites in

SEQ ID NO: 1:

5 4732 T;  
4813 G;  
6379 A;  
6762 G;  
7779 -;  
8058 C;  
8915 G;  
10 12228 C;  
9198 A and 5867 G;  
9198 A and 4800 C;  
3220 G and 5867 G; and  
3220 G and 4800 C  
15 or  
1386 C;  
2418 G;  
20 2583 T;  
3920 C;  
5867 G and 2405 C;  
5867 G and 4919 G;  
5867 G and 4956 C;  
25 5867 G and 6187 T;  
5867 G and 9534 C;  
5867 G and 12109 C;  
4800 C and 2405 C;  
4800 C and 4919 G;  
30 4800 C and 4956 C;  
4800 C and 6187 T;  
4800 C and 9534 C; and  
4800 C and 12109 C.

35 Non-risk genotypes or protective genotypes may be selected alone or in combination from  
the following EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

40 6196 C;  
5515 C;  
4946 C;  
4054 C;  
3402 C;  
3063 A; and  
2973 T.

The above identified sequence positions refer to the sense strand of the protein C gene and/or EPCR gene as indicated. It will be obvious to a person skilled in the art that analysis could be conducted on the anti-sense strand to determine patient outcome.

5

## **BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 shows shows haplotypes and haplotype clades of the protein C gene.

FIG. 2 shows haplotypes and haplotype clades of the endothelial cell protein C (EPCR) receptor gene.

10 FIG. 3 shows phylogenetic tree of EPCR haplotypes generated with MEGA2 software.

FIG. 4 shows Days Alive and Free of Acute Lung Injury/ARDS by EPCR Haplotype Clade.

15 FIG. 5 shows a Kaplan-Meier curve of the survival of groups 1, 2 and 3 of the protein C/EPCR haplotypes over 28 days.

FIG. 6 shows a Kaplan-Meier curve of the survival by genotype of endothelial protein C receptor 4054 T/C in critically ill patients with SIRS over 28 days.

FIG. 7 shows a Kaplan-Meier curve of the survival by genotype of protein C 4732 T/C in critically ill patients with SIRS over 28 days.

20

## **DETAILED DESCRIPTION OF THE INVENTION**

### **1. Definitions**

In the description that follows, a number of terms are used extensively, the following definitions are provided to facilitate understanding of the invention.

25

"Genetic material" includes any nucleic acid and can be a deoxyribonucleotide or ribonucleotide polymer in either single or double-stranded form.

30 A "purine" is a heterocyclic organic compound containing fused pyrimidine and imidazole rings, and acts as the parent compound for purine bases, adenine (A) and guanine (G).

"Nucleotides" are generally a purine (R) or pyrimidine (Y) base covalently linked to a

pentose, usually ribose or deoxyribose, where the sugar carries one or more phosphate groups. Nucleic acids are generally a polymer of nucleotides joined by 3' 5' phosphodiester linkages. As used herein "purine" is used to refer to the purine bases, A and G, and more broadly to include the nucleotide monomers, deoxyadenosine-5' -phosphate and deoxyguanosine-5' -phosphate, as components of a polynucleotide chain.

A "pyrimidine" is a single-ringed, organic base that forms nucleotide bases, cytosine (C), thymine (T) and uracil (U). As used herein "pyrimidine" is used to refer to the pyrimidine bases, C, T and U, and more broadly to include the pyrimidine nucleotide monomers that along with purine nucleotides are the components of a polynucleotide chain.

A nucleotide represented by the symbol M may be either an A or C, a nucleotide represented by the symbol W may be either an T or A, a nucleotide represented by the symbol S may be either an G or C, while a nucleotide represented by the symbol R may be either an G or A.

A "polymorphic site" or "polymorphism site" or "polymorphism" or "single nucleotide polymorphism site" (SNP site) as used herein is the locus or position with in a given sequence at which divergence occurs. A "Polymorphism" is the occurrence of two or more forms of a gene or position within a gene (allele), in a population, in such frequencies that the presence of the rarest of the forms cannot be explained by mutation alone. The implication is that polymorphic alleles confer some selective advantage on the host. Preferred polymorphic sites have at least two alleles, each occurring at frequency of greater than 1%, and more preferably greater than 10% or 20% of a selected population. Polymorphism sites may be at known positions within a nucleic acid sequence or may be determined to exist using the methods described herein. Polymorphisms may occur in both the coding regions and the noncoding regions (for example, promoters and introns) of genes.

A "risk genotype" as used herein refers to an allelic variant (genotype) at one or more polymorphism sites within the Protein C gene or EPCR gene described herein as being indicative of a decreased likelihood of recovery from an inflammatory condition or an increased risk of having a poor outcome. The risk genotype may be determined for either

the haploid genotype or diploid genotype, provided that at least one copy of a risk allele is present. Such "risk alleles" or "risk genotype" may be selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

5	4732 C; 4813 A; 6379 G; 6762 A; 7779 C;
10	8058 T; 8915 T; 12228 T; 9198 C and 5867 A; 9198 C and 4800 G;
15	3220 A and 5867 A; and 3220 A and 4800 G
	or
20	1386 T; 2418 A; 2583 A; 3920 T; 5867 A and 2405 T;
25	5867 A and 4919 A; 5867 A and 4956 T; 5867 A and 6187 C; 5867 A and 9534 T; 5867 A and 12109 T;
30	4800 G and 2405 T; 4800 G and 4919 A; 4800 G and 4956 T; 4800 G and 6187 C; 4800 G and 9534 T;
35	4800 G and 12109 T; 9198 A and 6379 G and 2405 T; 9198 A and 6379 G and 4919 A; 9198 A and 6379 G and 4956 T;
40	9198 A and 6379 G and 6187 C; 9198 A and 6379 G and 9534 T; and 9198 A and 6379 G and 12109 T.

Or such "risk alleles" or "risk genotype" may be selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

45	6196 G; 5515 T;
----	--------------------

4946 T;  
4054 T;  
3402 G;  
3063 G; and  
2973 C.

5

Furthermore, protein C (4732; 2418 and single or combined polymorphism sites in total linkage disequilibrium or having a high degree of linkage disequilibrium  $D' \geq 0.8$ ) and EPCR 4054 and single or combined polymorphism sites in total linkage disequilibrium or having a high degree of linkage disequilibrium ( $D' \geq 0.8$ ) "risk alleles" or "risk genotypes" may be combined to improve the predictive value and to improve the determinative value in deciding whether to treat a patient or mammal with an anti-inflammatory agent or an anti-coagulant agent.

10

A "clade" is a group of haplotypes that are closely related phylogenetically. For example, if haplotypes are displayed on a phylogenetic (evolutionary) tree a clade includes all haplotypes contained within the same branch.

15

As used herein "haplotype" is a set of alleles of closely linked loci on a chromosome that tend to be inherited together; commonly used in reference to the linked genes of the major histocompatibility complex. "Haplotypes" are also represented as rows in the Table represented in Figures 1 and 2.

20

As used herein "linkage disequilibrium" is the occurrence in a population of certain combinations of linked alleles in greater proportion than expected from the allele frequencies at the loci. Accordingly, if the genotype of a first locus corresponds to a second locus (or third locus etc.), the identification of only one locus would necessarily provide the identity of the other locus, provided the loci were in "total linkage disequilibrium" or have a high degree of linkage disequilibrium (i.e. an absolute value for  $D'$  of  $\geq 0.8$  or  $r^2 \geq 0.8$ ). Alternatively, a high degree of linkage disequilibrium may be represented by an absolute value for  $D'$  of  $\geq 0.85$  or  $r^2 \geq 0.85$  or by an absolute value for  $D'$  of  $\geq 0.9$  or  $r^2 \geq 0.9$ . The terms "complete linkage disequilibrium" or "total linkage disequilibrium" are used to refer to a population association between two loci that are linked wherein the two or more loci (SNPs) always have the same corresponding genotypes. However, two SNPs that have a high degree of linkage disequilibrium (i.e.

25

30

LD'  $\geq$  0.8) are also useful in determining the genotype of a corresponding SNP.

Therefore, we assume that a tested SNP is representative of another SNP in "total linkage disequilibrium" or having a high degree of linkage disequilibrium. For example, in the population from which the haplotype map was created the SNP at position 4054 of SEQ.

5 ID NO.: 2 was in "total linkage disequilibrium" with position 6196 of SEQ. ID NO.: 2, whereby when the genotype of 4054 is T the genotype of 6196 is G. Similarly, when the genotype of 4054 is C the genotype of 6196 is C. Accordingly, the determination of the genotype of a single locus will necessarily provide the identity of the genotype of any locus in "total linkage disequilibrium" therewith and is likely to provide the identity of the  
10 genotype of any locus having a high degree of linkage disequilibrium thereto.

The "promoter" region is 5' or upstream of the translation start site, in this case the translation start site is located at position 4062 of TABLE 1A (SEQ. ID NO: 1) and the transcription start site is located at position 2559 of TABLE 1A (SEQ. ID NO: 1).

15

Numerous sites have been identified as polymorphism sites in the EPCR gene, where those polymorphisms are linked to the polymorphism at position 4054 of SEQ. ID NO: 2 and may also therefore be indicative of patient prognosis. The following single polymorphism sites are linked to 4054 of SEQ. ID NO.: 2:

20

6196;  
5515;  
4946;  
3402;  
3063; and  
25 2973.

25

It will be appreciated by a person of skill in the art that further linked single polymorphism sites and combined polymorphism sites could be determined. The haplotype of protein C or EPCR can be created by assessing the SNPs of protein C and/or EPCR in normal  
30 subjects using a program that has an expectation maximization algorithm (i.e. PHASE). A constructed haplotype of protein C and/or EPCR may be used to find combinations of SNP's that are in total linkage disequilibrium (LD) with 4732 of SEQ ID NO: 1 and/or 4054 of SEQ ID NO: 2. Therefore, the haplotype of an individual could be determined by genotyping other SNPs that are in total LD with 4732 of SEQ ID NO: 1 and/or 4054 of

SEQ ID NO: 2. Linked single polymorphism sites or combined polymorphism sites may also be genotyped for assessing patient prognosis.

Previously identified single nucleotide polymorphisms in the protein C gene were  
5 described in international patent application, PCT/CA03/00751, which is incorporated  
herein by reference. Polymorphism sites in the protein C gene previously identified  
correspond to position 2418 of SEQ ID NO.: 1 or polymorphism sites in total linkage  
disequilibrium thereto. Such polymorphism may also be used as risk genotypes alone or  
in combination with other Protein C or EPCR gene risk genotypes in determining a  
10 patient's suitability for administration the anti-inflammatory agent or the anti-coagulant  
agent.

The following genotypes for single polymorphism sites in SEQ ID NO: 2 may indicative  
of a decreased likelihood of recovery from an inflammatory condition or indicative of  
15 severe cardiovascular or respiratory dysfunction in critically ill patients (risk alleles or risk  
genotypes):

20 6196 G;  
5515 T;  
4946 T;  
4054 T;  
3402 G;  
3063 G; and  
2973 C.

25 Whereas the following genotypes for single polymorphism sites in SEQ ID NO: 2 may  
indicative of a increased likelihood of recovery from an inflammatory condition or  
indicative of less severe cardiovascular or respiratory dysfunction in critically ill patients  
(protective alleles or protective genotypes):

30 6196 C;  
5515 C;  
4946 C;  
4054 C;  
3402 C;  
3063 A; and  
35 2973 T.

It will be appreciated by a person of skill in the art, that the numerical designations of the positions of polymorphisms within a sequence are relative to the specific sequence. Also the same positions may be assigned different numerical designations depending on the way in which the sequence is numbered and the sequence chosen, as illustrated by the  
5 alternative numbering of equivalent polymorphisms in Foster *et al.* and Millar *et al.* above. Furthermore, sequence variations within the population, such as insertions or deletions, may change the relative position and subsequently the numerical designations of particular nucleotides at and around a polymorphism site.

TABLE 1A below is representative of a *Homo sapiens* protein C gene sequence and comprises a sequence as listed in GenBank under accession number AF378903. The SNPs described as -1654 C/T, -1641 A/G and -1476 A/T using the numbering system of Foster *et al.* correspond to 2405, 2418 and 2583 respectively in TABLE 1A (SEQ ID NO:1. Polymorphism sites shown below in TABLE 1A are shown in bold and are capitalized. The major and minor alleles for each of the 4732 and linked polymorphism sites of the protein C gene are as follows (combination sites are not given here):

at position 4732 the most common nucleotide (major allele) is t and the minor allele is c;

at position 4813 the most common nucleotide (major allele) is g and the minor allele is a;

at position 6379 the most common nucleotide (major allele) is a and the minor allele is g;

at position 9198 the most common nucleotide (major allele) is a and the minor allele is c;

at position 5867 the most common nucleotide (major allele) is g and the minor allele is a;

at position 4800 the most common nucleotide (major allele) is c and the minor allele is g;

at position 3220 the most common nucleotide (major allele) is g and the minor allele is a.

**TABLE 1A**

1	gctctctaac	tcacagcgag	ctcgctgcc	aaagtcctgc	tccgggggct	tccgtgggtg
61	acctgaccgc	gttcgggtgc	acgtggggcg	actcacacct	gacaagtaaa	gcgggtgagg
5	121	ccgcgcctgt	gaagggcgcc	tggctcctcc	gcaggacggt	gcggcgcggc
181	ggaaccaggt	gtaactgcag	agaccctggg	atcgaggaa	cggctggcgg	caggactgtc
241	cctacctcga	gaaggtgacg	gggtttcctg	cgtgccagc	cgatgaggcg	gccgtgacgc
301	agcccgcctg	gcagagtccc	cgtcggccga	caggcgtgca	gagctctgca	gaggaccctt
361	ccgccctctg	ggcagcctgc	caagcctggg	cacccccaac	cccagcact	gggcacttgg
10	421	gagcattgca	gccgccctgg	ctcgtaaccg	tgccggtgct	ttgggcacct
481	ggacatgggt	gccccgggca	gagtcatttt	atgcagggtca	gaatcagtgt	gtggagcctg
541	catagacttg	ccctggagcg	gctgcctgtg	ctggggtggg	gaggagtaga	gggcgagaag
601	ttggtgggga	aggggaagcg	cgccaaaaga	ataccacaa	catcttgac	ctggaaggca
661	aagcagaggg	cagtgatctc	tgcagacttg	cgggggcgac	gcctgaagca	aacagggaca
15	721	tacaagctgg	tgccctctgt	ggttgtgcat	gggtcttca	tgcttctgt
781	agaagcttgt	ctctgctttt	ctaggcagct	gccacagcct	gtcacaaca	gctcctgggt
841	ctccacttct	catagtctcg	atttcaaaat	ccattgcctc	accctccacc	tccctccacc
901	ctccaccct	cctagcacct	cctgactgct	tgtgttctgt	gtctccccc	tgtctcccaa
961	cctggggtgg	ggttgggggg	gatgtctttc	ctcctgtctg	ctctttgatg	tccagctgaa
20	1021	gtgtcacctc	ctacaggcag	cctcccctgg	ctatgccagc	ttgtactgat
1081	tctgaattct	gtaagcattt	cctatgtgta	cctgcccctg	ggcaagggtg	gcctgacttg
1141	ttagagtgtt	agagtttttac	cctgttctc	taggagggcc	tggtaccacc	acagcccagc
1201	atggtgtggt	gcctcagcag	gaggcatctg	gttacaatca	acacaagctg	ttccagccaa
1261	tttaaagaaa	cttcaggagg	aatagggttt	taggagggca	tggggaccct	cctgcacccg
25	1321	aagccaggat	gtgccaccaa	tcataaggag	gcaggggcct	ccttccgctg
1381	ctctcYaggt	gtccgtggcc	tcagccccc	tctgcacacc	tgcatcttcc	ttctcatcag
1441	cttctctctg	tttaagcgta	aacatggatg	cccaggacct	ggcctcaatc	ttccgagtct
1501	ggtacttatg	gtgtactgac	agtgtgagac	cctactcctc	tgatcaatcc	cctgggttgg
1561	tgacttccct	gtgcaatcaa	tggaagccag	cgaggcaggg	tcacatgccc	cgttttagagg
30	1621	tgcagacttg	gagaaggaa	gtgggcaagt	cttcccagga	acaggtaggg
1681	aggggggcat	ctctggtgca	gcccggttcg	gagcaggaag	acgcttaata	aatgctgata
1741	gactgcagga	cacaggcaaa	ggtgctgagc	tggacccttt	atttctgccc	ttctcccttc
1801	tggcaccocg	gccaggaaat	tgtctgagcc	tttctggaat	cccgttcatt	tttcttactg
1861	gtccacaaaa	ggggccaaat	ggaagcagca	agacctgagt	tcaaattaaa	tctgccaaact
35	1921	accagctcag	tgaatctggg	cgagtaacac	aaaacttgag	tgtccttacc
1981	aggttagagg	gatgctatgt	gccattgtgt	gtgtgtgttg	ggggtgggga	ttgggggtga
2041	tttgtgagca	attggaggtg	aggggtggagc	ccagtgccca	gcacctatgc	actggggacc
2101	caaaaaggag	catcttctca	tgatatttatg	tatcagaaat	tgggatggca	tgtcattggg
2161	acagcgtctt	ttttcttgta	tgggtggcaca	taaatacatg	tgtcttataa	ttaatgggtat

2221 tttagatttg acgaaatatg gaatattacc tgttggtgctg atcttgggca aactataata  
 2281 tctctgggca aaaatgtccc catctgaaaa acagggacaa cgttcctccc tca<sup>g</sup>gccagcc  
 2341 actatggggc taaaatgaga ccacatctgt caagggtttt gccctcacct ccctccctgc  
 2401 tggaYggcat ccttgg<sup>R</sup>g cagaggtggg ctctgggcag aacaagccgt gctgagctag  
 5 2461 gaccaggagt gctagtgcc ctgtttgtct atggagaggg aggcctcagt gctgagggcc  
 2521 aagcaaatat ttgtggttat ggattaactc gaactccagg ctgtcatggc ggcaggacgg  
 2581 cgWacttgca gtatctccac gaccgcccc tgtgagtcct cctccaggca ggtctatgag  
 2641 ggggtgtggag ggagggctgc ccccgggaga agagagctag gtgggtgatga gggctgaatc  
 2701 ctccagccag ggtgctcaac aagcctgagc ttggggtaa aggacacaag gccctccaca  
 10 2761 ggccaggcct ggcagccaca gtctcaggtc cttttgccat ggcctccct ctttccaggc  
 2821 caagggtccc cagggccag gccattcca acagacagtt tggagcccag gacctccat  
 2881 tctccccacc ccacttccac ctttgggggt gtcggtattg aacaaatctc agaagcggcc  
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 4021 agctcagaag tctcctcag acaggtgcca gtgcctccag aatgtggcag ctcaacagcc  
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 10 5161 cagggaggcc tgtccatctc agagcaaggc ttctcctcc aactgccatc tgcttctctg  
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 7681 gggccactg ttagcgcaat cagcccgga gctggcgcg ccctccgctt tccctgcttc  
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 7801 tggagcgcaa gccagtggt ggctccgct cccagtctga gcgtatctgg ggcgaggcgt  
 15 7861 gcagcgtcct cctccatgta gcctggctgc gtttttctt gacgttgtcc ggcgtgcac  
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 8581 ctgggattac aggcgtgagc caccgcgcc agcctctttc agggaaactt ctacaacttt  
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 35 9061 gctggtgcct gtgcaggctg tggacatttg catgactccc tgtggtcagc taagagcacc  
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 12901 tccttcacg gcacctggtt cctggtgggc ctggtgagct ggggtgaggg ctgtgggctc  
 20 12961 cttcacaact acggcgttta caccaaagtc agccgctacc tcgactggat ccattgggac  
 13021 atcagagaca aggaagcccc ccagaagagc tgggcacctt agcgaccctc cctgcagggc  
 13081 tgggcttttg catggcaatg gatgggacat taaagggaca tgtaacaagc acaccggcct  
 13141 gctgttctgt ccttccatcc ctcttttggg ctcttctgga gggaagtaac atttactgag  
 13201 cacctgttgt atgtcacatg ctttatgaat agaattctaa ctcttagagc aactctgtgg  
 25 13261 ggtggggagg agcagatcca agttttgcgg ggtctaaagc tgtgtgtgtt gagggggata  
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 13801 taagtcaagg atgctctgat ttgaaatcat gaagtacctg atgaaaagaa atggtggtga  
 35 13861 gataaagctg

TABLE 1B below is representative of a Human endothelial cell protein C receptor (EPCR) gene sequence (SEQ ID NO:2). Polymorphism sites shown below in TABLE 1B are shown in bold and capitalized. The major and minor alleles for each of the primary polymorphism sites of the EPCR gene are as follows:

- 5           at position 6196 the most common nucleotide (major allele) is **g** and the minor allele is **c**;  
            at position 5515 the most common nucleotide (major allele) is **t** and the minor allele is **c**;  
            at position 4946 the most common nucleotide (major allele) is **t** and the minor  
10          allele is **c**;  
            at position 4054 the most common nucleotide (major allele) is **t** and the minor allele is **c**;  
            at position 3402 the most common nucleotide (major allele) is **g** and the minor allele is **c**;  
15          at position 3063 the most common nucleotide (major allele) is **g** and the minor allele is **a**;  
            at position 2973 the most common nucleotide (major allele) is **c** and the minor allele is **t**.

TABLE 1B

	tagagaagcg	agaccacatc	tctagtaaaa	ataaaaaaaaa	aatagctagg	50
	cgtggtggca	cagtggcacg	tacctttagt	ctcagctact	cgggtggttg	100
5	aggtgggaga	atcacttgag	cccgggaggt	caagcctaca	attagctgtg	150
	attgcttcac	tgcactatag	cctgggcaac	agagctagac	cctgtctcaa	200
	aaaaataata	ataaatTTTa	tatatatata	tgaggatgaa	attacatatg	250
	tattatttga	acagaagtga	aatcttttct	tttttttttt	caaaaaaat	300
	tttgccgcat	gccccaggct	aaaatgcagt	ggtgtgatct	gggccctctg	350
10	aaacctccac	ctcccgggtt	caagggattc	tcatgcctcg	gtctcccaag	400
	tagctgggat	tacaggcatg	caccaccatg	cccagctaata	ttttgtattt	450
	ttcgtagaga	cgttcgccat	attggccagg	ctggtctcaa	actcctggcc	500
	tcaagtgate	tgcccacctc	ggcctcccaa	agtgccagca	gcatgctcgg	550
	aggagtgact	ttaaagcttt	tctacttgct	tcctagagta	agggacgcat	600
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	aagcacacat	atacaactga	gcaaatattt	catgacataa	cacttttctct	700
	tactaagggg	gacgcgctga	aattttgtat	tctgtcctat	ttcatttttt	750
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20	agatttatac	agattgctca	taattctctc	ctatttttta	aaaacatgct	900
	gcagtgaact	gctttacact	cattttatga	ctacttctga	gaccaagatc	950
	ccggattatg	taattgttat	ttacttaaaa	ttctggtaaa	atgtagccat	1000
	tatactggaa	aactaaattt	taatcttgga	tctgtcacca	ccatgatata	1050
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25	gcaacctgct	gatcctactc	ccaggagtgt	gctctaagtt	gaaagtagat	1150
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	ccccttttcc	gctccctggt	cctggttcct	aggaagcagc	ccaaggagaa	1250
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	gagggcagga	gggaggccgg	ccccctagta	ggaaatgaga	cacagtagaa	1350
30	ataacacttt	ataagcctct	tctctctccc	atctcctggc	ctccttccat	1400
	cctcctctgc	ccagactccg	cccctcccag	acggtcctca	cttctctttt	1450
	ccctagactg	cagccagcgg	agcccgcagc	cggcccgcagc	caggaaccca	1500
	ggtccggagc	ctcaacttca	ggatggtgac	aacattgctg	ccgatactgc	1550
	tgctgtctgg	ctgggccttt	tgtagccaag	acgcctcaga	tggtgagtcg	1600
35	ggggcacatc	tcctgcctca	ggatggttct	ggagaatctc	agtctatctg	1650
	ggcacatggc	aagaccacag	gagagcttat	ctcacagcat	ctgtgtctgc	1700
	agctggctag	atctctctac	agggcaggca	gagtcttggg	gactggttcg	1750
	tgtcccaaag	ccaaggtag	ttagtacatt	taagcccctg	aaaaggggga	1800
	gatgaaagag	gctaggggaa	acaggatgac	tggaacatg	agaaagaaac	1850
40	cagcagagag	ggtaggagaa	tcagccccag	ggagagggga	gaaaggggaa	1900
	ctgaggggtga	tggtagatag	gggtacatct	aggggagacg	ggaagaggct	1950
	cagaagagaa	gagaaatgga	gggaatggga	agaccctggg	aaaactgatg	2000
	gaagaagtgg	gggaagagtg	gggcagagag	aggttagggg	aggctaggga	2050
	aaatggaagg	agactggtcg	cagctgggtg	aactggggag	aaagagatgc	2100
45	tgtgcctaata	agaacttatg	ggcgatcagg	ctactgaagt	ggccctgttt	2150
	aagcagaaaa	gggagttatt	accctccatt	ataattgcac	aggggcctcc	2200
	tttcccctct	ctcacaatcc	ccgtaacttc	agtctccccc	tcagagaggc	2250
	agcaaataat	aaccagtatt	caatgagtgc	tcactatggt	taatacatgt	2300
	attgacccat	ttaacttgca	caaaccctca	aagggtggga	atattattac	2350

	tatctccatt	ttatgaggag	gaaactgggt	cacagagtag	ttaaggacca	2400
	tgtctagggt	tatccataaa	tatacttatt	cacatctgca	gatacaaagc	2450
	acaacttctc	aaatgcaaac	acagacagga	cccactcaca	cacacagatt	2500
	tacaaccccg	gactcatcca	aatgtgctct	gggcatcaac	tctgtgccag	2550
5	cctcttttct	gggtgtagga	agcagagatt	accaagcatg	gttccatagc	2600
	ctagaggagt	ccagtgtggc	ctgtgtgtgt	ttggagacag	ccaggtagta	2650
	tcccgtgaga	tacacactaa	tatatggtgg	tctgggatca	ctgaaacaga	2700
	cacactgtgt	ctcgtggggc	atcagaaaaa	aatttccaag	aagagggcaa	2750
	ctgagctggg	tctttttttc	tttgcttttc	tttctttttt	cttttttttt	2800
10	tttttttttt	tttttgagat	ggagtcttgt	gctgtcacc	aggctggaat	2850
	gcagtggcac	aatttcagct	aactgtaacc	tccaactccc	aggttcaggc	2900
	gattctcctg	cctcagcctc	ctgagtagct	gggactacag	gcatgtacca	2950
	ccacgcctgg	ctaataattg	taYttttagt	acagatgggg	tttcgccatg	3000
	ttggccaggc	tgggtcttgaa	tccctgacct	caagtgatcc	gcccgcctcg	3050
15	gcctcccaaa	gtRctgggat	tacaggcatg	agccaccgcg	cccagtctct	3100
	gagctgggtc	ttaaatacatg	aataaacttc	gccaggcaga	aaaagggagg	3150
	cagagcaatc	ctgacatgct	attcatgtgt	cagccaaagg	cagcatgagg	3200
	aatcccaact	agtttgatat	ataagcagcg	ggaagcggcc	agaaaaggca	3250
	gcaggggcca	ggtctctagc	agccttgaat	gccaggctaa	agactctgga	3300
20	cttgatcctg	tggggaggca	gtgtagcaga	atggctgagt	gctggacttg	3350
	actgcctacg	tgcaaacctt	ggctctgcta	cactatctct	gtctcagttt	3400
	cScatgtaga	ctgggggttaa	taatagtagc	tattgcatta	agccactggg	3450
	gaaaggcaca	aagataataa	tgtatgtaaa	gcccattgcc	caggttataa	3500
	taagcactga	atcgacattg	gctatgatta	tttttgatta	atgaagggga	3550
25	gggggttatg	gcaactggaag	attttaagta	ggaaaaggac	atgatctcat	3600
	ccctgggtca	ggtggaggtc	ggaatagaga	acggggagat	gaagtagaaa	3650
	gttactaccc	cagtctagat	gagacggatg	aatcctgaat	cagggcagtg	3700
	gaagaggaga	tggagaacag	gcgatggaat	tggaaattta	ttcaggtcag	3750
	gatttggttaa	ccatttgttc	cgttgggttaa	caggaaacgg	ggggagggag	3800
30	agccgagggg	gaaaaaggag	gcagaaagga	gtgtctcttc	cactgcaggc	3850
	ctcagtttcc	tcatctgtaa	aacggagata	ataatccctg	tcctgtcctc	3900
	ctggcagagt	tactgtcagc	gtcaaacggg	agaagcggtg	ggagggcaca	3950
	ttatagttta	tgaagggctg	agaagggcgg	cggccagcct	cgaggtaggg	4000
	ggttattatc	ttccgctgcc	cgcgcggccc	tcccacgccc	gcccaggctg	4050
35	aagYtgactc	tgcccgcagg	cctccaaaga	cttcatatgc	tccagatctc	4100
	ctacttccgc	gacccctatc	acgtgtggta	ccagggcaac	gcgtcgctgg	4150
	ggggacacct	aacgcacgtg	ctggaaggcc	cagacaccaa	caccacgatc	4200
	attcagctgc	agcccttgca	ggagcccag	agctgggcgc	gcacgcagag	4250
	tggcctgcag	tcctacctgc	tccagttcca	cggcctcgtg	cgccctggtg	4300
40	accaggagcg	gaccttgccc	tgtgagtagg	cgcgacgcgc	gggcgggggc	4350
	tgggcggggc	tagtgggggc	ggggcctggc	gggtgggggc	ggggcctggc	4400
	ggatggaggc	gggctggggc	ttgcaggggc	ccggcagcca	ctggagctcg	4450
	gtggcgccctg	ggcctttgaa	gattgctggg	tgggggctgg	agagaggcag	4500
	ttgtccccgc	taagaaagcc	ccgactcggg	cggtcgtcct	gctggcataa	4550
45	cctcttgggg	tagaccctgt	tgggaaggcc	tgacaccgtg	acgtcgaagg	4600
	tccccagaaa	actcctcacc	cctcgcctca	cagtcctcca	actccttttc	4650
	ttcatagatc	tccgtccttc	ccttcccaca	gccccagca	cttcaccccc	4700
	caccctccag	ccacttctca	tacaagctga	tgacttcgct	cttagctcca	4750
	ctcatgaccc	gaactcttcc	cccaaagacc	ccaagttctt	ctctcaaagc	4800

	cccactcctt	ccccgtcaca	accctaactc	cttctttctca	aagaccccaa	4850
	tttcttttct	caaagcacca	agcaccactc	cgccccctt	ccccaccat	4900
	catggccttt	aactccttct	tctcctagtc	ccccaccca	ccccYtttt	4950
	tttttttttt	tttttttttt	gagacggagt	cttgctctgt	cgccagggt	5000
5	ggagtgcagt	ggcgcgatct	cggtcactg	caacttccgc	ctcccggtt	5050
	caagcgattc	tcctgcctca	gcctcccaag	cagctgggac	tacaggcacc	5100
	cgccaccacg	cccggctaata	tttttgatt	tttagtagag	acgggggttc	5150
	gccatgttgg	ccaggctggg	ctcgaactcc	tgacctcagg	cgatccacaa	5200
	gcctggcctc	ccaaagtgt	gggattacag	gcgtgagctg	ccgcccctgc	5250
10	cccagcctca	ccccctgttt	tttttttcta	ttacagttga	acaaggcctg	5300
	acaattccct	tttttcatca	cagtccctgg	ccccttcttt	cttagcctct	5350
	aacaggctaa	ccccaaaccc	ctcctcacag	ccccaggccc	ttctcccat	5400
	agttccctga	cctagactcc	cctctcctca	cagcactgac	tcttgccctc	5450
	tcatgttctt	ttccccttgg	tgggcctcgc	ccacacctgg	cacctctct	5500
15	gcacagtccc	ctgaYcctga	ctgtctatcc	acagtccctc	tgacctccg	5550
	ctgcttctctg	ggctgtgagc	tgctcccgga	gggctctaga	gcccattgtct	5600
	tcttcgaagt	ggctgtgaat	gggagctcct	ttgtgagttt	ccggccggag	5650
	agagccttgt	ggcaggcaga	caccaggtc	acctccggag	tggtcacctt	5700
	cacctgcag	cagctcaatg	cctacaaccg	cactcggtat	gaactgcggg	5750
20	aattcctgga	ggacacctgt	gtgcagtatg	tgcaaaaaca	tatttccgcg	5800
	gaaaacacga	aaggtatgat	gggacggggc	ccaggcctgc	aagctgggga	5850
	gagggcgggt	tccagacaaa	tggatggacc	tgaaggatgg	atgcctagag	5900
	caacaagagg	cccacagctg	ggggtttggg	acagaacaca	cgcagcttca	5950
	gtcagttggg	aaacgggtcc	ctttcctctg	gggcagaaac	gctttggggg	6000
25	ttgactcaaa	tcatggactc	cttggggggc	tattcttcgg	gctaactctt	6050
	tgcatgttct	gcagggagcc	aaacaagccg	ctcctacact	tcgctgggtc	6100
	tgggcgtcct	ggtgggcagt	ttcatcattg	ctgggtgtggc	tgtaggcac	6150
	ttcctgtgca	cagggtggacg	gcgatgttaa	ttactctcca	gccccStcag	6200
	aaggggctgg	attgatggag	gctggcaagg	gaaagtittca	gctcactgtg	6250
30	aagccagact	ccccaaactga	aacaccagaa	ggtttgaggt	gacagctcct	6300
	ttctttctccc	acatctgccc	actgaagatt	tgaggagggg	gagatggaga	6350
	ggagagggtg	acaaagtact	tggtttgcta	agaacctaaag	aacgtgtatg	6400
	ctttgctgaa	ttagtctgat	aagtgaatgt	ttatctatct	ttgtggaaaa	6450
	cagataatgg	agttggggca	ggaagcctat	ggcccatcct	ccaaagacag	6500
35	acagaatcac	ctgaggcggt	caaaagatat	aaccaaataa	acaagtcac	6550
	cacaatcaaa	atacaacatt	caatacttcc	aggtgtgtca	gacttgggat	6600
	gggacgctga	tataataggg	tagaaagaag	taacacgaag	aagtgggtga	6650
	aatgtaaaat	ccaagtcata	tggcagtgat	caattattaa	tcaattaata	6700
	atattaataa	atttcttata	tttaaggcat	tggtatctcc	tccactttgc	6750
40	aaaattttctg	gaaaagtaac	ctatacccat	ttcttctgct	tccttatttc	6800
	tcaactattc	tttttttttt	tttttttttt	tttgagacag	agtcttgctc	6850
	tggtgcctag	gctggagtg	aatgggtgtga	tctcagctca	ctgcaacctc	6900
	tgctcccg	ttcaagcaat	tctcctgcct	cagcctccca	agcagctggg	6950
	attacagatg	catgccacca	caccagcta	atttttgat	ttttagtaga	7000
45	gatgggggtt	caccacgttg	gccatcctga	cctcgtgatc	cgcctacctc	7050
	ggcctcccca	agtgtctggga	ttagacgtga	gccactgcgc	ctgggtcttct	7100
	cactcattct	tagaccaggt	gcaatctgac	ttctctataa	actactctga	7150
	gatcaccagt	aacctctaata	tgtcaaacca	tcacctaca	tggtatctg	

**TABLE 1C**

The sequences shown in TABLE 1C, are sequence fragments taken from the protein C sequence shown in TABLE 1A above. Furthermore, SEQ ID NO.: 3 corresponds to the sequence underlined in TABLE 1A above. The nucleotide Y, at position 8 in SEQ ID NO.: 3 corresponds to the nucleotide found at position 4732 of SEQ ID NO.: 1. In all of the Sequences found in TABLE 1C below the polymorphism represented by a Y may substituted by an t or c. Furthermore, **bold** and underlined nucleotides represented by Y in SEQ ID NOs.: 4-12 in TABLE 1C, all correspond to the nucleotide found at position 4732 of SEQ ID NO.: 1. Due to the potential variability in protein C sequence, the sequence motifs below may be useful in identifying protein C sequences from a patient that are suitable for genotype determination. For Example, patient sequences that form alignments with the below motifs (SEQ ID NO.: 3-12) may indicate that the patient sequence is a protein C sequence and that the **bold** and underlined Y corresponds to the polymorphism at position 4732 of SEQ ID NO.: 1 and is therefore suitable for genotype determination. A similar strategy may be applied to the other polymorphism sites identified herein.

SEQ ID. NO.	SEQUENCE
SEQ ID. NO. 3	gccttt <u>Y</u> cc cccatccctt
SEQ ID. NO. 4	aggatgcctt t <u>Y</u> cccccatc
SEQ ID. NO. 5	<u>Y</u> cccccatcc cttcttgctc
SEQ ID. NO. 6	<u>Y</u> cccccatcc cttcttgctc acacccccaa
SEQ ID. NO. 7	cctcctctag gatgccttt <u>Y</u>
SEQ ID. NO. 8	Tcaggcatac cctcctctag gatgccttt <u>Y</u>
SEQ ID. NO. 9	gctcaggcat accctcctct aggatgcctt t <u>Y</u>
SEQ ID. NO. 10	gctcaggcat accctcctct aggatgcctt t <u>Y</u> cccccatc ccttcttgct cacacccccca acttgatctc tccctcctaa
SEQ ID. NO. 11	aggatgcctt t <u>Y</u>
SEQ ID. NO. 12	gccttt <u>Y</u> ccc ccatcccttc

**TABLE 1D**

The sequences shown in TABLE 1D, are sequence fragments taken from the EPCR sequence shown in TABLE 1B above. Furthermore, SEQ ID NO.: 13 corresponds to the sequence underlined in TABLE 1B above. The nucleotide S, at position 8 in SEQ ID NO.:

- 5 13 corresponds to the nucleotide found at position 6196 of SEQ ID NO.: 2. In all of the sequences found in TABLE 1D below the polymorphism represented by an S may substituted by a "g" or "c". Furthermore, **bold** and underlined nucleotides represented by S in SEQ ID NOs.: 14-22 in TABLE 1D, all correspond to the nucleotide found at position 6196 of SEQ ID NO.: 2. Due to the potential variability in EPCR sequence, the sequence
- 10 motifs below may be useful in identifying EPCR sequences from a patient that are suitable for genotype determination. For Example, patient sequences that form alignments with the below motifs (SEQ ID NO.: 13-22) may indicate that the patient sequence is an EPCR sequence and that the **bold** and underlined S corresponds to the polymorphism at position 6196 of SEQ ID NO.: 2 and is therefore suitable for genotype determination. A similar
- 15 strategy may be applied to the other polymorphism sites identified herein.

SEQ ID. NO.	SEQUENCE
SEQ ID. NO. 13	cagcccc <u>S</u> tc agaaggggct
SEQ ID. NO. 14	tctccagccc c <u>S</u> tcagaagg
SEQ ID. NO. 15	<u>S</u> tcagaagg gctggattga
SEQ ID. NO. 16	<u>S</u> tcagaagg gctggattga tggaggctgg
SEQ ID. NO. 17	ttaattactc tccagcccc <u>S</u>
SEQ ID. NO. 18	gacggcgatg ttaattactc tccagcccc <u>S</u>
SEQ ID. NO. 19	gcgatgttaa ttactctcca gcccc <u>S</u> tcag aaggggctgg attgatggag
SEQ ID. NO. 20	tgtaggcatc ttcctgtgca caggtggacg gcgatgttaa ttactctcca gcccc <u>S</u> tcag aaggggctgg attgatggag gctggcaagg gaaagtttca
SEQ ID. NO. 21	tctccagccc c <u>S</u>
SEQ ID. NO. 22	agcccc <u>S</u> tca gaaggggctg

TABLE 1E

db SNP #	Gene	Chromosome/ Contig Accession	SNP	Location Relative to Gene
rs908787	PROC	2 NT_005079	[C/G]	5'
rs777566	PROC	2 NT_005079	[C/G]	3'
rs334135	PROC	2 NT_005079	[T/C]	3'
rs777569	PROC	2 NT_005079	[A/T]	3'
rs334142	PROC	2 NT_005079	[A/G]	3'
rs334160	PROC	2 NT_005079	[T/C]	3'
rs334159	PROC	2 NT_005079	[T/C]	3'
rs334151	PROC	2 NT_005079	[C/T]	3'
rs334146	PROC	2 NT_005079	[C/A]	3'
rs777556	PROC	2 NT_005079	[C/T]	3'
rs334144	PROC	2 NT_005079	[A/C]	3'
rs2295887	EPCR	20 NT_028392	[A/G]	5'
rs1535466	EPCR	20 NT_028392	[A/G]	5'
rs1033797	EPCR	20 NT_028392	[C/T]	5'
rs1033798	EPCR	20 NT_028392	[C/T]	5'
rs1033799	EPCR	20 NT_028392	[A/C]	5'
rs2295888	EPCR	20 NT_028392	[A/G]	5'
rs666210	EPCR	20 NT_028392	[C/T]	5'
rs1415771	EPCR	20 NT_028392	[A/G]	5'
rs945959	EPCR	20 NT_028392	[C/G]	5'
rs1051056	EPCR	20 NT_028392	[A/C]	3'
rs632688	EPCR	20 NT_011387	[A/C]	3'
rs633198	EPCR	20 NT_011387	[C/T]	3'
rs663550	EPCR	20 NT_011387	[A/T]	3'

TABLE 1E shows are "rs" identifier number for each of the SNPs identified herein as lying outside of the protein C and EPCR sequences (SEQ ID NOS:1 and 2 respectively).

- 5 The data base SNP # (db SNP # - have a "rs" prefix designates a SNP in the database is found at the NCBI SNP database

(<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp>).

The "rs" numbers are the NCBI | rsSNP ID form. Also shown are the chromosome and contig accession numbers on which the sequences may be found, the alleles at each SNP

- 10 and the position of the SNP relative to the protein C or EPCR gene.

TABLE 1F

db SNP #	Gene	SNP	SNP including flanking sequence
rs908787	PROC	[C/G]	gcagaggacccttc[c/g]gccctctgggcagcc SEQ ID NO.:23
rs777566	PROC	[C/G]	tcctctggaaacag[c/g]ccctccttcatat

rs334135	PROC	[T/C]	SEQ ID NO.:24 cacagccaaaaaag[t/c]gtgaacacaa
rs777569	PROC	[A/T]	SEQ ID NO.:25 tggggaactcactc[a/t]atttccatgctatctct
rs334142	PROC	[A/G]	SEQ ID NO.:26 tttcagttatatcc[a/g]tatttccttga
rs334160	PROC	[T/C]	SEQ ID NO.:27 tagggtcattat[t/c]gaaactaaaagcagacctgg
rs334159	PROC	[T/C]	SEQ ID NO.:28 acctctcgtgtata[t/c]actctggtagggcc
rs334151	PROC	[C/T]	SEQ ID NO.:29 gaagcgaccagct[c/t]acctcagcagcttca
rs334146	PROC	[C/A]	SEQ ID NO.:30 gacaaatctcttga[c/a]atcagtatatggctggttt
rs777556	PROC	[C/T]	SEQ ID NO.:31 ttgcagttttatta[c/t]gatgtagttaggtgtagatt
rs334144	PROC	[A/C]	SEQ ID NO.:32 ttttgtgtataata[a/c]gtacatatgaaaaacttaaa
rs2295887	EPCR	[A/G]	SEQ ID NO.:33 tatactctgcagtg[a/g]gggagatgggataatggaca
rs1535466	EPCR	[A/G]	SEQ ID NO.:34 ggaacataagatga[a/g]taaggcatggattctgcatt
rs1033797	EPCR	[C/T]	SEQ ID NO.:35 agatgcagggcagg[c/t]gccccagtgcttcttgggaa
rs1033798	EPCR	[C/T]	SEQ ID NO.:36 caccagcatgtga[c/t]tccactatctgaagacacag
rs1033799	EPCR	[A/C]	SEQ ID NO.:37 ctgacagagtgggt[a/c]taaggagagaaaccgaatag
rs2295888	EPCR	[A/G]	SEQ ID NO.:38 tctttctcctgggt[a/g]tcctgctagagtctgagcca
rs666210	EPCR	[C/T]	SEQ ID NO.:39 agagatttcctctc[c/t]gggcctaaagggtcaaacaac
rs1415771	EPCR	[A/G]	SEQ ID NO.:40 gtaagaattgcggg[a/g]agcgcggtcttagctcagct
rs945959	EPCR	[C/G]	SEQ ID NO.:41 aaagggaaaggacc[c/g]ggttcacgcttcccattccc
rs1051056	EPCR	[A/C]	SEQ ID NO.:42 taaacaagtcattcc[a/c]caatcaaaatacaacattca
rs632688	EPCR	[A/C]	SEQ ID NO.:43 cccacccaaacaaa[a/c]aacaaaaccattatatttat
rs633198	EPCR	[C/T]	SEQ ID NO.:44 agattagatttggt[c/t]tgtggaattccaggggaacag
rs663550	EPCR	[A/T]	SEQ ID NO.:45 acattaaaaaaaaa[a/t]tatttgtttaggggtctgtcc
			SEQ ID NO.:46

TABLE 1F shows are "rs" identifier number for each of the SNPs identified in TABLE 1E along with flanking sequences of each SNP.

An "allele" is defined as any one or more alternative forms of a given gene. In a diploid cell or organism the members of an allelic pair (i.e. the two alleles of a given gene) occupy corresponding positions (loci) on a pair of homologous chromosomes and if these alleles are genetically identical the cell or organism is said to be "homozygous", but if genetically different the cell or organism is said to be "heterozygous" with respect to the particular gene.

A "gene" is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product and may include untranslated and untranscribed sequences in proximity to the coding regions. Such non-coding sequences may contain regulatory sequences needed for transcription and translation of the sequence or introns etc.

A "genotype" is defined as the genetic constitution of an organism, usually in respect to one gene or a few genes or a region of a gene relevant to a particular context (i.e. the genetic loci responsible for a particular phenotype).

A "phenotype" is defined as the observable characters of an organism.

A "single nucleotide polymorphism" (SNP) occurs at a polymorphic site occupied by a single nucleotide, which is the site of variation between allelic sequences. The site is usually preceded by and followed by highly conserved sequences of the allele (e.g., sequences that vary in less than 1/100 or 1/1000 members of the populations). A single nucleotide polymorphism usually arises due to substitution of one nucleotide for another at the polymorphic site. A "transition" is the replacement of one purine by another purine or one pyrimidine by another pyrimidine. A "transversion" is the replacement of a purine by a pyrimidine or vice versa. Single nucleotide polymorphisms can also arise from a deletion (represented by "-" or "del") of a nucleotide or an insertion (represented by "+" or "ins") of a nucleotide relative to a reference allele. Furthermore, it would be appreciated by a person of skill in the art, that an insertion or deletion within a given sequence could alter the relative position and therefore the position number of another polymorphism within the sequence.

A "systemic inflammatory response syndrome" or (SIRS) is defined as including both septic (i.e. sepsis or septic shock) and non-septic systemic inflammatory response (i.e. post operative). "SIRS" is further defined according to ACCP (American College of Chest Physicians) guidelines as the presence of two or more of A) temperature  $> 38^{\circ}\text{C}$  or  $< 36^{\circ}\text{C}$ , B) heart rate  $> 90$  beats per minute, C) respiratory rate  $> 20$  breaths per minute, and D) white blood cell count  $> 12,000$  per  $\text{mm}^3$  or  $< 4,000$   $\text{mm}^3$ . In the following description, the presence of two, three, or four of the "SIRS" criteria were scored each day over the 28 day observation period.

"Sepsis" is defined as the presence of at least two "SIRS" criteria and known or suspected source of infection. Septic shock was defined as sepsis plus one new organ failure by Brussels criteria plus need for vasopressor medication.

Patient outcome or prognosis as used herein refers the ability of a patient to recover from an inflammatory condition. An inflammatory condition, may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumonia, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased

risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglottitis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystic carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

Assessing patient outcome or prognosis may be accomplished by various methods. For Example, an "APACHE II" score is defined as Acute Physiology And Chronic Health Evaluation and herein was calculated on a daily basis from raw clinical and laboratory variables. Vincent *et al.* (Vincent JL, Ferreira F, Moreno R. *Scoring systems for assessing organ dysfunction and survival*. Critical Care Clinics. 16:353-366, 2000) summarize APACHE score as follows "First developed in 1981 by Knaus *et al.*, the APACHE score has become the most commonly used survival prediction model in ICUs worldwide. The APACHE II score, a revised and simplified version of the original prototype, uses a point score based on initial values of 12 routine physiologic measures, age, and previous health status to provide a general measure of severity of disease. The values recorded are the worst values taken during the patient's first 24 hours in the ICU. The score is applied to one of 34 admission diagnoses to estimate a disease-specific probability of mortality (APACHE II predicted risk of death). The maximum possible APACHE II score is 71, and high scores have been well correlated with mortality. The APACHE II score has been widely used to stratify and compare various groups of critically ill patients, including patients with sepsis, by severity of illness on entry into clinical trials." Furthermore, the criteria or indication for administering activated protein C (XIGRIS™ -drotrecogin alfa

(activated)) in the United States is an APACHE II score of  $\geq 25$ . In Europe, the criteria or indication for administering activated protein C is an APACHE II score of  $\geq 25$  or 2 organ system failures.

5 “Activated protein C” is also known as Drotrecogin alfa (activated) and is sold as XIGRIS™ by Eli Lilly and Company. Drotrecogin alfa (activated) is a serine protease glycoprotein of approximately 55 kilodalton molecular weight and having the same amino acid sequence as human plasma-derived Activated Protein C. The protein consists of a heavy chain and a light chain linked by a disulfide bond. XIGRIS™, Drotrecogin alfa  
10 (activated) is indicated for the reduction of mortality in adult patients with severe sepsis (sepsis associated with acute organ dysfunction) who have a high risk of death (e.g., as determined by an APACHE II score of greater  $> 25$  or having 2 or more organ system failures).

15 XIGRIS™ is available in 5 mg and 20 mg single-use vials containing sterile, preservative-free, lyophilized drug. The vials contain 5.3 mg and 20.8 mg of drotrecogin alfa (activated), respectively. The 5 and 20 mg vials of XIGRIS™ also contain 40.3 and 158.1 mg of sodium chloride, 10.9 and 42.9 mg of sodium citrate, and 31.8 and 124.9 mg of sucrose, respectively. XIGRIS™ is recommended for intravenous administration at an  
20 infusion rate of 24 mcg/kg/hr for a total duration of infusion of 96 hours. Dose adjustment based on clinical or laboratory parameters is not recommended. If the infusion is interrupted, it is recommended that when restarted the infusion rate should be 24 mcg/kg/hr. Dose escalation or bolus doses of drotrecogin alfa are not recommended. XIGRIS™ may be reconstituted with Sterile Water for Injection and further diluted with  
25 sterile normal saline injection. These solutions must be handled so as to minimize agitation of the solution (Product information. XIGRIS™, Drotrecogin alfa (activated), Eli Lilly and Company, November 2001).

30 Drotrecogin alfa (activated) is a recombinant form of human Activated Protein C, which may be produced using a human cell line expressing the complementary DNA for the inactive human Protein C zymogen, whereby the cells secrete protein into the fermentation medium. The protein may be enzymatically activated by cleavage with thrombin and subsequently purified. Methods, DNA compounds and vectors for producing recombinant

activated human protein C are described in US patents 4,775,624; 4,992,373; 5,196,322; 5,270,040; 5,270,178; 5,550,036; 5,618,714 all of which are incorporated herein by reference.

5 Treatment of sepsis using activated protein C in combination with a bactericidal and  
endotoxin neutralizing agent is described in US patent 6,436,397; methods for processing  
protein C is described in US patent 6,162,629; protein C derivatives are described in US  
patents 5,453,373 and 6,630,138; glycosylation mutants are described in US patent  
5,460,953; and Protein C formulations are described in US patents 6,630,137, 6,436,397,  
10 6,395,270 and 6,159,468, all of which are incorporated herein by reference.

A "Brussels score" score is a method for evaluating organ dysfunction as compared to a  
baseline. If the Brussels score is 0 (ie. moderate, severe, or extreme), then organ failure  
was recorded as present on that particular day (see TABLE 2A below). In the following  
15 description, to correct for deaths during the observation period, days alive and free of  
organ failure (DAF) were calculated as previously described. For example, acute lung  
injury was calculated as follows. Acute lung injury is defined as present when a patient  
meets all of these four criteria. 1) Need for mechanical ventilation, 2) Bilateral pulmonary  
infiltrates on chest X-ray consistent with acute lung injury, 3)  $\text{PaO}_2/\text{FiO}_2$  ratio is less than  
20 300, 4) No clinical evidence of congestive heart failure or if a pulmonary artery catheter is  
in place for clinical purposes, a pulmonary capillary wedge pressure less than 18 mm Hg  
(1). The severity of acute lung injury is assessed by measuring days alive and free of  
acute lung injury over a 28 day observation period. Acute lung injury is recorded as  
present on each day that the person has moderate, severe or extreme dysfunction as  
25 defined in the Brussels score. Days alive and free of acute lung injury is calculated as the  
number of days after onset of acute lung injury that a patient is alive and free of acute lung  
injury over a defined observation period (28 days). Thus, a lower score for days alive and  
free of acute lung injury indicates more severe acute lung injury. The reason that days  
alive and free of acute lung injury is preferable to simply presence or absence of acute  
30 lung injury, is that acute lung injury has a high acute mortality and early death (within 28  
days) precludes calculation of the presence or absence of acute lung injury in dead  
patients. The cardiovascular, renal, neurologic, hepatic and coagulation dysfunction were  
similarly defined as present on each day that the person had moderate, severe or extreme

dysfunction as defined by the Brussels score. Days alive and free of steroids are days that a person is alive and is not being treated with exogenous corticosteroids (e.g. hydrocortisone, prednisone, methylprednisolone). Days alive and free of pressors are days that a person is alive and not being treated with intravenous vasopressors (e.g. dopamine, norepinephrine, epinephrine, phenylephrine). Days alive and free of an International Normalized Ratio (INR) > 1.5 are days that a person is alive and does not have an INR > 1.5.

**TABLE 2A**

**Brussels Organ Dysfunction Scoring System**

ORGANS	Free of Organ Dysfunction		Clinically Significant Organ Dysfunction		
	Normal	Mild	Moderate	Severe	Extreme
<b>DAF ORGAN DYSFUNCTION SCORE</b>	<b>1</b>		<b>0</b>		
<u>Cardiovascular</u> Systolic BP (mmHg)	>90	≤90 Responsive to fluid	≤90 Unresponsive to fluid	≤90 plus pH ≤7.3	≤90 plus pH ≤7.2
<u>Pulmonary</u> P <sub>a</sub> O <sub>2</sub> /F <sub>I</sub> O <sub>2</sub> (mmHg)	>400	400-301	300-201 Acute lung injury	200-101 ARDS	≤100 Severe ARDS
<u>Renal</u> Creatinine (mg/dL)	<1.5	1.5-1.9	2.0-3.4	3.5-4.9	≥5.0
<u>Hepatic</u> Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
<u>Hematologic</u> Platelets (x10 <sup>5</sup> /mm <sup>3</sup> )	>120	120-81	80-51	50-21	≤20
<u>Neurologic</u> (Glasgow Score)	15	14-13	12-10	9-6	≤5
Round Table Conference on Clinical Trials for the Treatment of Sepsis Brussels, March 12-14, 1994.					

Analysis of variance (ANOVA) is a standard statistical approach to test for statistically significant differences between sets of measurements.

The Fisher exact test is a standard statistical approach to test for statistically significant differences between rates and proportions of characteristics measured in different groups.

## 2. General Methods

One aspect of the invention may involve the identification of patients or the selection of patients that are either at risk of developing and inflammatory condition or the

5 identification of patients who already have an inflammatory condition. For example, patients who have undergone major surgery or scheduled for or contemplating major surgery may be considered as being at risk of developing an inflammatory condition. Furthermore, patients may be determined as having an inflammatory condition using

10 diagnostic methods and clinical evaluations known in the medical arts. An inflammatory condition, may be selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumonia, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-

15 reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected

20 endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis,

25 patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure,

30 hepatitis, epiglottitis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystis carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme

disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

Once a patient is identified as being at risk for developing or having an inflammatory condition, then genetic sequence information may be obtained from the patient. Or alternatively genetic sequence information may already have been obtained from the patient. For example, a patient may have already provided a biological sample for other purposes or may have even had their genetic sequence determined in whole or in part and stored for future use. Genetic sequence information may be obtained in numerous different ways and may involve the collection of a biological sample that contains genetic material. Particularly, genetic material, containing the sequence or sequences of interest. Many methods are known in the art for collecting bodily samples and extracting genetic material from those samples. Genetic material can be extracted from blood, tissue and hair and other samples. There are many known methods for the separate isolation of DNA and RNA from biological material. Typically, DNA may be isolated from a biological sample when first the sample is lysed and then the DNA is isolated from the lysate according to any one of a variety of multi-step protocols, which can take varying lengths of time. DNA isolation methods may involve the use of phenol (Sambrook, J. *et al.*, "Molecular Cloning", Vol. 2, pp. 9.14-9.23, Cold Spring Harbor Laboratory Press (1989) and Ausubel, Frederick M. *et al.*, "Current Protocols in Molecular Biology", Vol. 1, pp. 2.2.1-2.4.5, John Wiley & Sons, Inc. (1994)). Typically, a biological sample is lysed in a detergent solution and the protein component of the lysate is digested with proteinase for 12-18 hours. Next, the lysate is extracted with phenol to remove most of the cellular components, and the remaining aqueous phase is processed further to isolate DNA. In another method, described in Van Ness *et al.* (U.S. Pat. # 5,130,423), non-corrosive phenol derivatives are used for the isolation of nucleic acids. The resulting preparation is a mix of RNA and DNA.

Other methods for DNA isolation utilize non-corrosive chaotropic agents. These methods, which are based on the use of guanidine salts, urea and sodium iodide, involve lysis of a biological sample in a chaotropic aqueous solution and subsequent precipitation of the crude DNA fraction with a lower alcohol. The final purification of the precipitated, crude DNA fraction can be achieved by any one of several methods, including column chromatography (Analects, (1994) Vol 22, No. 4, Pharmacia Biotech), or exposure of the crude DNA to a polyanion-containing protein as described in Koller (U.S. Pat. # 5,128,247).

Yet another method of DNA isolation, which is described by Botwell, D. D. L. (Anal. Biochem. (1987) 162:463-465) involves lysing cells in 6M guanidine hydrochloride, precipitating DNA from the lysate at acid pH by adding 2.5 volumes of ethanol, and washing the DNA with ethanol.

Numerous other methods are known in the art to isolate both RNA and DNA, such as the one described by Chomczynski (U.S. Pat. # 5,945,515), whereby genetic material can be extracted efficiently in as little as twenty minutes. Evans and Hugh (U.S. Pat. # 5,989,431) describe methods for isolating DNA using a hollow membrane filter.

Once a patient's genetic sequence information has been obtained from the patient it may then be further analyzed to detect or determine the identity or genotype of one or more polymorphisms in the protein C gene. Provided that the genetic material obtained, contains the sequence of interest. Particularly, a person may be interested in determining the protein C genotype of a patient of interest, where the genotype includes a nucleotide corresponding to position 4732 or SEQ ID NO.: 1 or position 8 of SEQ ID NO.: 3. The sequence of interest may also include other protein C gene polymorphisms or may also contain some of the sequence surrounding the polymorphism of interest. Detection or determination of a nucleotide identity or the genotype of the single nucleotide polymorphism(s) or other polymorphism, may be accomplished by any one of a number of methods or assays known in the art, including but not limited to the following:

Restriction Fragment Length Polymorphism (RFLP) strategy – An RFLP gel-based analysis can be used to distinguish between alleles at polymorphic sites within a gene. Briefly, a short segment of DNA (typically several hundred base pairs) is

amplified by PCR. Where possible, a specific restriction endonuclease is chosen that cuts the short DNA segment when one variant allele is present but does not cut the short DNA segment when the other allele variant is present. After incubation of the PCR amplified DNA with this restriction endonuclease, the reaction products are then separated using gel electrophoresis. Thus, when the gel is examined the appearance of two lower molecular weight bands (lower molecular weight molecules travel farther down the gel during electrophoresis) indicates that the initial DNA sample had the allele which could be cut by the chosen restriction endonuclease. In contrast, if only one higher molecular weight band is observed (at the molecular weight of the PCR product) then the initial DNA sample had the allele variant that could not be cut by the chosen restriction endonuclease. Finally, if both the higher molecular weight band and the two lower molecular weight bands are visible then the initial DNA sample contained both alleles, and therefore the patient was heterozygous for this single nucleotide polymorphism;

Sequencing – For example the Maxam-Gilbert technique for sequencing (Maxam AM. and Gilbert W. Proc. Natl. Acad. Sci. USA (1977) 74(4):560-564) involves the specific chemical cleavage of terminally labelled DNA. In this technique four samples of the same labeled DNA are each subjected to a different chemical reaction to effect preferential cleavage of the DNA molecule at one or two nucleotides of a specific base identity. The conditions are adjusted to obtain only partial cleavage, DNA fragments are thus generated in each sample whose lengths are dependent upon the position within the DNA base sequence of the nucleotide(s) which are subject to such cleavage. After partial cleavage is performed, each sample contains DNA fragments of different lengths, each of which ends with the same one or two of the four nucleotides. In particular, in one sample each fragment ends with a C, in another sample each fragment ends with a C or a T, in a third sample each ends with a G, and in a fourth sample each ends with an A or a G. When the products of these four reactions are resolved by size, by electrophoresis on a polyacrylamide gel, the DNA sequence can be read from the pattern of radioactive bands. This technique permits the sequencing of at least 100 bases from the point of labeling. Another method is the dideoxy method of sequencing was published by Sanger *et al.* (Sanger *et al.* Proc. Natl. Acad. Sci.

USA (1977) 74(12):5463-5467). The Sanger method relies on enzymatic activity of a DNA polymerase to synthesize sequence-dependent fragments of various lengths. The lengths of the fragments are determined by the random incorporation of dideoxynucleotide base-specific terminators. These fragments can then be separated in a gel as in the Maxam-Gilbert procedure, visualized, and the sequence determined. Numerous improvements have been made to refine the above methods and to automate the sequencing procedures. Similarly, RNA sequencing methods are also known. For example, reverse transcriptase with dideoxynucleotides have been used to sequence encephalomyocarditis virus RNA (Zimmern D. and Kaesberg P. Proc. Natl. Acad. Sci. USA (1978) 75(9):4257-4261). Mills DR. and Kramer FR. (Proc. Natl. Acad. Sci. USA (1979) 76(5):2232-2235) describe the use of Q.beta. replicase and the nucleotide analog inosine for sequencing RNA in a chain-termination mechanism. Direct chemical methods for sequencing RNA are also known (Peattie DA. Proc. Natl. Acad. Sci. USA (1979) 76(4):1760-1764). Other methods include those of Donis-Keller *et al.* (1977, Nucl. Acids Res. 4:2527-2538), Simoncsits A. *et al.* (Nature (1977) 269(5631):833-836), Axelrod VD. *et al.* (Nucl. Acids Res.(1978) 5(10):3549-3563), and Kramer FR. and Mills DR. (Proc. Natl. Acad. Sci. USA (1978) 75(11):5334-5338, which are incorporated herein by reference). Nucleic acid sequences can also be read by stimulating the natural fluoresce of a cleaved nucleotide with a laser while the single nucleotide is contained in a fluorescence enhancing matrix (U.S. Pat. # 5,674,743);

Hybridization methods for the identification of SNPs using hybridization techniques are described in the U.S. Pat. # 6,270,961 & 6,025,136;

A template-directed dye-terminator incorporation with fluorescent polarization-detection (TDI-FP) method is described by FREEMAN BD. *et al.* (J Mol Diagnostics (2002) 4(4):209-215) is described for large scale screening;

Oligonucleotide ligation assay (OLA) - is based on ligation of probe and detector oligonucleotides annealed to a polymerase chain reaction amplicon strand with detection by an enzyme immunoassay (VILLAHERMOSA ML. J Hum Virol

(2001) 4(5):238-48; ROMPPANEN EL. Scand J Clin Lab Invest (2001) 61(2):123-9; IANNONE MA. *et al.* Cytometry (2000) 39(2):131-40);

5 Ligation-Rolling Circle Amplification (L-RCA) has also been successfully used for genotyping single nucleotide polymorphisms as described in QI X. *et al.* Nucleic Acids Res (2001) 29(22):E116;

5' nuclease assay has also been successfully used for genotyping single nucleotide polymorphisms (AYDIN A. *et al.* Biotechniques (2001) (4):920-2, 924, 926-8.);

10 Polymerase proofreading methods are used to determine SNPs identities, as described in WO 0181631;

15 Detection of single base pair DNA mutations by enzyme-amplified electronic transduction is described in PATOLSKY F *et al.* Nat Biotech. (2001) 19(3):253-257;

20 Gene chip technologies are also known for single nucleotide polymorphism discrimination whereby numerous polymorphisms may be tested for simultaneously on a single array (EP 1120646 and Gilles PN. *et al.* Nat. Biotechnology (1999) 17(4):365-70);

25 Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectroscopy is also useful in the genotyping single nucleotide polymorphisms through the analysis of microsequencing products (Haff LA. and Smirnov IP. Nucleic Acids Res. (1997) 25(18):3749-50; Haff LA. and Smirnov IP. Genome Res. (1997) 7:378-388; Sun X. *et al.* Nucleic Acids Res. (2000) 28 e68; Braun A. *et al.* Clin. Chem. (1997) 43:1151-1158; Little DP. *et al.* Eur. J. Clin. Chem. Clin. Biochem. (1997) 35:545-548; Fei Z. *et al.* Nucleic Acids Res. (2000) 26:2827-2828; and Blondal T. *et al.* Nucleic Acids Res. (2003) 31(24):e155); or

Allele specific PCR methods have also been successfully used for genotyping single nucleotide polymorphisms (Hawkins JR. *et al.* Hum Mutat (2002) 19(5):543-553).

- 5 Alternatively, if a patient's sequence data is already known, then obtaining may involve retrieval of the patients nucleic acid sequence data from a database, followed by determining or detecting the identity of a nucleic acid or genotype at a polymorphism site by reading the patient's nucleic acid sequence at the polymorphic site.
- 10 Once the identity of a polymorphism(s) is determined or detected an indication may be obtained as to patient outcome or prognosis or ability of a patient recover from an inflammatory condition based on the genotype (the nucleotide at the position) of the polymorphism of interest. In the present invention, polymorphisms in protein C sequence and/or polymorphisms in endothelial cell protein C receptor (EPCR) sequence, are used to
- 15 obtain a prognosis or to determine patient outcome. Methods for obtaining patient outcome or prognosis or for patient screening may be useful to determine the ability of a patient to recover from an inflammatory condition. Alternatively, single polymorphism sites or combined polymorphism sites may be used as an indication of a patient's ability to recover from an inflammatory condition, if they are linked to a polymorphism determined
- 20 to be indicative of a patient's ability to recover from an inflammatory condition. The method may further comprise comparing the genotype determined for a polymorphism with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition. Accordingly, a decision regarding the patient's ability to recover may be from an
- 25 inflammatory condition may be made based on the genotype determined for the polymorphism site.

Once patient outcome or a prognosis is determined, such information may be of interest to physicians and surgeons to assist in deciding between potential treatment options, to help

30 determine the degree to which patients are monitored and the frequency with which such monitoring occurs. Ultimately, treatment decisions may be made in response to factors, both specific to the patient and based on the experience of the physician or surgeon responsible for a patient's care. Treatment options that a physician or surgeon may

consider in treating a patient with an inflammatory condition may include, but are not limited to the following:

- (a) use of anti-inflammatory therapy;
- (b) use of steroids;
- 5 (c) use of activated Protein C (drotrocogin alpha or Xigris™ from Lilly);
- (d) use of modulators of the coagulation cascade (such as various versions of heparin) use of antibody to tissue factor;
- (e) use of anti-thrombin or anti-thrombin III;
- 10 (f) streptokinase;
- (g) use of antiplatelet agents such as clopidogrel; and
- (h) Surfactant.

Alternative treatments currently in development and potentially useful in the treatment of an inflammatory condition may include, but are not limited to the following: antibodies to tumor necrosis factor (TNF) or even antibody to endotoxin (i.e. lipopolysaccharide, LPS); tumor necrosis factor receptor (TNF); tissue factor pathway inhibitors (tifacogin™ alpha from Chiron); platelet activating factor hydrolase (PAFase™ from ICOS); antibodies to IL-6; antibodies, antagonists or inhibitors to high mobility group box 1 (HMGB-1 or HMG-1 tissue plasminogen activator; bradykinin antagonists; antibody to CD-14; interleukin-10; Recombinant soluble tumor necrosis factor receptor-immunoglobulin G1(Roche); Procysteine; Elastase Inhibitor; and human recombinant interleukin 1 receptor antagonist (IL-1 RA).

25 Methods of treatment of an inflammatory condition in a patient having one or more of the risk genotypes in protein C and/or EPCR associated with improved response to a therapeutic agent are described herein. An improved response may include an improvement subsequent to administration of said therapeutic agent, whereby the patient has an increased likelihood of survival, reduced likelihood of organ damage or organ dysfunction (Brussels score), an improved APACHE II score, days alive and free of pressors, inotropes, and reduced systemic dysfunction (cardiovascular, respiratory, ventilation, CNS, coagulation [INR> 1.5], renal and/or hepatic).

As described above genetic sequence information or genotype information may be obtained from a patient wherein the sequence information contains one or more single nucleotide polymorphism sites in protein C sequence and/or EPCR sequence. Also, as previously described the sequence identity of one or more single nucleotide polymorphisms in the protein C sequence and EPCR sequence of one or more patients may then be detected or determined. Furthermore, patient outcome or prognosis may be assessed as described above, for example the APACHE II scoring system or the Brussels score may be used to assess patient outcome or prognosis by comparing patient scores before and after treatment. Once patient outcome or prognosis has been assessed, patient outcome or prognosis may be correlated with the sequence identity of one or more single nucleotide polymorphism(s). The correlation of patient outcome or prognosis may further include statistical analysis of patient outcome scores and polymorphism(s) for a number of patients.

#### **Clinical Phenotype**

The primary outcome variable was survival to hospital discharge. Secondary outcome variables were days alive and free of cardiovascular, respiratory, renal, hepatic, hematologic, and neurologic organ system failure as well as days alive and free of SIRS (Systemic Inflammatory Response Syndrome), occurrence of sepsis, and occurrence of septic shock. SIRS was considered present when patients met at least two of four SIRS criteria. The SIRS criteria were 1) fever ( $>38^{\circ}\text{C}$ ) or hypothermia ( $<35.5^{\circ}\text{C}$ ), 2) tachycardia ( $>100$  beats/min in the absence of beta blockers, 3) tachypnea ( $>20$  breaths/min) or need for mechanical ventilation, and 4) leukocytosis (total leukocyte count  $> 11,000/\mu\text{L}$ ) (Anonymous. *Critical Care Medicine* (1992) 20(6):864-74). Patients were included in this cohort on the calendar day on which the SIRS criteria were met.

A patients' baseline demographics that were recorded included age, gender, whether medical or surgical diagnosis for admission (according to APACHE III diagnostic codes (KNAUS WA et al. *Chest* (1991) 100(6):1619-36)), and admission APACHE II score. The following additional data were recorded for each 24 hour period (8 am to 8 am) for 28 days to evaluate organ dysfunction, SIRS, sepsis, and septic shock.

Clinically significant organ dysfunction for each organ system was defined as present during a 24 hour period if there was evidence of at least moderate organ dysfunction using the Brussels criteria (TABLE 2A) (RUSSELL JA et al. *Critical Care Medicine* (2000) 28(10):3405-11). Because data were not always available during each 24 hour period for each organ dysfunction variable, we used the "carry forward" assumption as defined previously (Anonymous. *New England Journal of Medicine* (2000) 342(18):1301-8). Briefly, for any 24 hour period in which there was no measurement of a variable, we carried forward the "present" or "absent" criteria from the previous 24 hour period. If any variable was never measured, it was assumed to be normal.

To further evaluate cardiovascular, respiratory, and renal function we also recorded, during each 24 hour period, vasopressor support, mechanical ventilation, and renal support, respectively. Vasopressor use was defined as dopamine  $> 5 \mu\text{g/kg/min}$  or any dose of norepinephrine, epinephrine, vasopressin, or phenylephrine. Mechanical ventilation was defined as need for intubation and positive airway pressure (i.e. T- piece and mask ventilation were not considered ventilation). Renal support was defined as hemodialysis, peritoneal dialysis, or any continuous renal support mode (e.g. continuous veno-venous hemodialysis).

To assess duration of organ dysfunction and to correct organ dysfunction scoring for deaths in the 28-day observation period, calculations were made of days alive and free of organ dysfunction (DAF) as previously reported (BERNARD GR et al. *New England Journal of Medicine* (1997) 336(13):912-8). Briefly, during each 24-hour period for each variable, DAF was scored as 1 if the patient was alive and free of organ dysfunction (normal or mild organ dysfunction, Table 2A). DAF was scored as 0 if the patient had organ dysfunction (moderate, severe, or extreme) or was not alive during that 24-hour period. Each of the 28 days after ICU admission was scored in each patient in this fashion. Thus, the lowest score possible for each variable was zero and the highest score possible was 28. A low score is indicative of more organ dysfunction as there would be fewer days alive and free of organ dysfunction.

Similarly, days alive and free of SIRS (DAF SIRS) were calculated. Each of the four SIRS criteria were recorded as present or absent during each 24 hour period. Presence of SIRS during each 24 hour period was defined by having at least 2 of the 4 SIRS criteria. Sepsis was defined as present during a 24 hour period by having at least two of four SIRS  
5 criteria and having a known or suspected infection during the 24 hour period (Anonymous. *Critical Care Medicine* (1992) 20(6):864-74). Cultures that were judged to be positive due to contamination or colonization were excluded. Septic shock was defined as presence of sepsis plus presence of hypotension (systolic blood pressure < 90 mmHg or need for vasopressor agents) during the same 24 hour period.

10

#### **Haplotypes and Selection of htSNPs**

Using unphased Caucasian genotypic data (from [pga.mbt.washington.edu](http://pga.mbt.washington.edu) (RIEDER MJ et al. SeattleSNPs. NHLBI Program for Genomic Applications, UW-FHCRC, Seattle, WA (2001)) haplotypes were inferred using PHASE<sup>1</sup> (STEPHENS M. et al. *Am J Hum Genet*  
15 (2001) 68:978-89) software (Figures 1 and 2). MEGA 2 (KUMAR S. et al. (2001) 17:1244-5) was then used to infer a phylogenetic tree to identify major haplotype clades for EPCR (Figures 3). Haplotypes were sorted according to the phylogenetic tree and haplotype structure was inspected to choose haplotype tag SNPs (htSNPs) (JOHNSON GC. et al. *Nat Genet* (2001) 29:233-7; and GABRIEL SB. et al. *Science* (2002) 296:2225-  
20 9). htSNPs that identified major haplotype clades of EPCR in Caucasians were chosen. These SNPs were then genotyped in our patient cohort to define haplotypes and haplotype clades.

#### **Blood Collection/Processing Genotyping**

25 The buffy coat was extracted from whole blood and samples transferred into 1.5 ml cryotubes and stored at -80°C. DNA was extracted from the buffy coat of peripheral blood samples using a QIAamp DNA Blood Maxi Kit (Qiagen<sup>TM</sup>). The genotypic analysis was performed in a blinded fashion, without clinical information. Polymorphisms were genotyped using either a Masscode tagging (Qiagen Genomics, Inc - KOKORIS M et al  
30 *Molecular Diagnosis* (2000) 5(4):329-40; BRAY MS. et al. *Hum Mutat* (2001) 17:296-304.).

### **Data Collection**

Data was recorded for 28 days or until hospital discharge. Raw clinical and laboratory variables were recorded using the worst or most abnormal variable for each 24 hour period with the exception of Glasgow Coma Score, where the best possible score for each 24  
5 hour period was recorded. Missing data on the date of admission was assigned a normal value and missing data after the day one was substituted by carrying forward the previous day's value. Demographic and microbiologic data were recorded. When data collection for each patient was complete, all patient identifiers were removed from all records and the patient file was assigned a unique random number that was cross referenced with the  
10 blood samples. The completed raw data file was converted to calculated descriptive and severity of illness scores using standard definitions (i.e. APACHE II and Days alive and free of organ dysfunction calculated using the Brussels criteria).

### **Statistical Analysis**

15 We used a cohort study design. Rates of dichotomous outcomes (28-day mortality, sepsis and shock at onset of SIRS) were compared between haplotype clades using a chi-squared test, assuming a dominant model of inheritance. Differences in continuous outcome variables between haplotype clades were tested using ANOVA. 28-day mortality was further compared between haplotype clades while adjusting for other confounders (age,  
20 sex, and medical vs. surgical diagnosis) using a Cox regression model, together with Kaplan-Meier analysis. Haplotype clade relative risk was calculated. This analysis was performed in the entire cohort, and subsequently in sub-groups of patients who had sepsis at onset of SIRS, and patients who had septic shock at onset of SIRS. Genotype distributions were tested for Hardy-Weinberg equilibrium (GUO SW. and THOMPSON  
25 EA. (1992) 48:361-72). We report the mean and 95% confidence intervals. Statistical significance was set at  $p < 0.05$ . The data was analyzed using SPSS 11.5 for Windows™ and SigmaStat 3.0 software (SPSS Inc, Chicago, IL, 2003).

## **3. EXAMPLES**

30

### **EXAMPLE 1: EPCR Haplotype Analysis**

### Inclusion Criteria

498 consecutive critically ill patients admitted to St. Paul's Hospital Intensive Care Unit (ICU) met the inclusion criteria of having at least two out of four SIRS criteria and were included into our study.

Seven haplotypes of the EPCR gene were inferred using PHASE software as described above and phylogenetic analysis was used to sort these haplotypes into 3 clades (Figure 3). The htSNPs A6118G (rs867186) and G6196C (rs9574) to uniquely identify each haplotype clade (Figure 2). 222 Caucasian patients admitted to our ICU with SIRS and successfully genotyped for the A6118G and G6196C polymorphisms were included in this study. The genotype frequencies of A6118G and G6196C are shown in Table 3A. These alleles were in Hardy Weinberg equilibrium in our population. Haplotype clade 1, defined by 6118A/6196C, occurred with a frequency of 37%. Haplotype clade 2, defined by 6118A/6196G, occurred in 39% of our cohort, while haplotype clade 3, defined by 6118G/6196G, occurred in 24% of our cohort.

**TABLE 3A**  
Genotype frequencies of EPCR haplotype tag SNPs A6118G and C6196G

	Genotype Frequencies			Allele Frequencies		p*
	AA	AG	GG	A	G	
A6118G	81%	19%	0%	90.5%	9.5%	0.99
G6196C	CC	CG	GG	C	G	
	23%	41%	36%	44%	56%	0.98

\* Chi-Squared test for Hardy-Weinberg equilibrium

**TABLE 3B**  
Genotype frequencies of EPCR haplotype tag SNP T4054C

	Genotype Frequencies			Allele Frequencies		p*
	TT	CT	CC	T	C	
T4054C	30%	50%	20%	55%	45%	0.99

\*Chi-Squared test for Hardy-Weinberg equilibrium

5 **TABLE 3C**  
Baseline characteristics of critically ill patients who had systemic inflammatory response syndrome by genotype of endothelial protein C receptor T4054C.

Baseline Characteristics	4054 TT	4054 CT	4054 CC	p Value
N	151 (30%)	248 (50%)	99 (20%)	0.999*
Age	55 ± 1	58 ± 1	59 ± 2	0.095
Gender (% Male)	29	52	19	0.509
APACHE II	23 ± 1	23 ± 1	22 ± 1	0.372
Med/Surg	29	48	24	0.543
Sepsis Anytime	31	51	18	0.091
Septic Shock Anytime	30	52	18	0.435

\*Exact test for Hardy-Weinberg Equilibrium of Guo and Thompson.

10 The frequency of the alleles is shown in **Table 3C**. These alleles were in Hardy Weinberg equilibrium in our population. There were no significant differences in baseline characteristics of patients who had endothelial protein C receptor 4054 T/C and 6118 A/G (**Table 3C**). Patients were of similar age, similar gender distribution, and had similar admitting APACHE II scores. Approximately eighty percent of these patients developed sepsis and 45% of these patients developed septic shock at some time during their ICU stay.

20 **Table 4** shows that there were no significant differences in baseline characteristics of associated with haplotype clades 1, 2, or 3. Patients were of similar age had similar APACHE II scores. There was a trend to more males in haplotype 3 (**Table 4**). There was no difference in the frequency of sepsis or septic shock at the time of onset of SIRS (**Table 4**).

**TABLE 4**  
Baseline characteristics and mortality of 222 critically ill patients who had SIRS

Haplotype Clade	Mean Age	Gender (% Male)	Diagnosis for admission (% Surgical)	Mean APACHE II	Sepsis on Admission	Septic Shock On Admission	28-day Mortality
1	61	63%	30%	20	54%	43%	31%
2	59	65%	31%	19	61%	50%	37%
3	63	79%	33%	20	60%	52%	33%
p	NS	0.06	NS	NS	NS	NS	NS

The EPCR haplotype clades 2 and 3 were associated with fewer days alive and free of acute lung injury /ARDS injury than haplotype clade 1 (Figure 4) in our entire cohort of patients with SIRS. There was also a trend ( $p < 0.07$ ) to more acute renal dysfunction (expressed as fewer days alive and free of acute renal dysfunction) in haplotype clades 2 and 3. These associations were not seen in sub-groups of patients with sepsis at onset of SIRS, or those patients with septic shock at onset of SIRS.

There was no difference between between haplotype clades 1, 2 or 3 in 28 day mortality (Table 4). There were no associations of EPCR haplotypes with cardiovascular, neurologic, hepatic or coagulation dysfunction (Table 5A). There was also no association of haplotype or genotype with days alive and free of ventilatory, vasopressor or renal support (Table 5B).

**TABLE 5A**  
Days alive and free of (DAF) SIRS and Key Organ Dysfunction in 222 critically ill patients who had SIRS

Haplotype	DAF SIRS 4/4	DAF SIRS 3/4	DAF ALI	DAF CNS	DAF CVS	DAF COAG	DAF RENAL	DAF HEPATIC
1	22.	22	20	21	21	24	19	20
2	20	20	17	19	19	24	17	19
3	21	21	18	20	20	25	18	20
p	NS	NS	0.006	NS	NS	NS	0.07	NS

**TABLE 5B**  
Days alive and free of (DAF) Life Support in 222 critically ill patients who had SIRS

Haplotype	DAF Vasopressors	DAF Renal Support	DAF Ventilatory Support
1	19	19	15
2	18	18	14
3	19	19	15
p	NS	NS	NS

When examined individually, it was found that neither htSNP was associated with a  
 5 difference in baseline characteristics (age, sex, medical vs. surgical diagnosis, APACHE II  
 score), 28-day mortality, or days alive and free of organ dysfunction, with the exception of  
 acute lung injury. The EPCR 6196 G/G genotype was associated with significantly fewer  
 days alive and free of acute lung injury/ARDS than the 6196G/C and C/C genotypes  
 combined (16 days vs. 20 days,  $p < 0.006$ ), again indicating more acute lung injury/ARDS.  
 10 The 6196 G allele is contained within both haplotype clades 2 and 3.

In Figure 2 EPCR 4054 SNP corresponds to EPCR 6196 above. The EPCR 4054 T allele  
 is contained within both haplotype clades 2 and 3 and the C allele is contained within  
 clade 1. Similar results were found for patients who had the T allele of EPCR 4054 as for  
 15 the 6196 G allele. 28-day survival is shown in Figure 6. Patients with the endothelial  
 protein C receptor 4054 TT or TC genotype had a survival of 64 %, while those with the  
 CC genotype had a 71 % survival ( $p = 0.21$ ). Patients with the endothelial protein C  
 receptor 4054 T allele (TC and TT) had significantly more acute lung injury and strong  
 trend to greater mechanical ventilation as reflected by fewer days alive and free of acute  
 20 lung injury and mechanical ventilation (Table 6). Patients who had the T allele of  
 endothelial protein C receptor 4054 (TT and CT) also had more cardiovascular  
 dysfunction as shown by having significantly more days alive and free of cardiovascular  
 dysfunction and a trend to more days alive and free of vasopressor use (Table 6). Patients  
 who had the T allele of 4054 T/C also had more neurological dysfunction and had a trend  
 25 to more renal dysfunction (Table 6). Furthermore, the severity of the systemic  
 inflammatory response syndrome was greater in patients who had the endothelial protein

C receptor 4054 T allele as indicated by fewer days alive and free of four of four SIRS criteria (Table 6). Thus, the endothelial protein C receptor 4054 T allele was associated with more acute lung injury and need for mechanical ventilation, worse cardiovascular and neurologic dysfunction, more severe systemic inflammatory response syndrome and trends to worse renal function.

**TABLE 6**

Days Alive and Free (DAF) of organ dysfunction and severe systemic inflammatory response syndrome (4 out of 4 criteria) by 4054 T/C genotype of endothelial protein C receptor in critically ill patients who had systemic inflammatory response syndrome.

Days Alive and Free	Endothelial protein C receptor 4054 TT	Endothelial protein C receptor 4054 CT and CC	p Value
Acute Lung Injury	16±11.4	19.1±11.6	0.023*
Mechanical Ventilation	12.5±11.5	15±11.7	0.058
Cardiovascular	15.5±11.2	18.1±10.9	0.029
Vasopressor Use	17.6±11.3	19.7±10.9	0.086
CNS	18.3±11.2	20.3±10.8	0.014
Renal	16.7±11.8	19±11.4	0.081
SIRS 4 of 4	18.5±10.5	20.2±10.4	0.052

\* Significance tested by Spearman's Rho correlation.

#### EXAMPLE 2: Patient Outcome or Prognosis for 4732 Protein C

##### Polymorphisms

Table 7 shows the genotype frequencies of T4732C. These alleles were in Hardy Weinberg equilibrium in our population.

**TABLE 7**

Genotype frequencies of ProC haplotype tag SNP T4732C

	Genotype Frequencies			Allele Frequencies		p*
	TT	CT	CC	T	C	
T4732C	57%	37%	6%	76%	24%	0.99

\*Chi-Squared test for Hardy-Weinberg equilibrium

It was found that SNP haplotypes of protein C 4732 are associated with altered survival and organ dysfunction in critically ill adults who have systemic inflammatory response syndrome (SIRS).

5 For Tables 8A and 8B below an inception cohort of 489 Caucasian patients were studied in ICU who met at least 2/4 criteria for SIRS and defined subgroups of patients who had sepsis or septic shock. Baseline variables were age, gender, APACHE II and medical vs. surgical reason for ICU admission. We determined 28-day survival (Kaplan Meier) and scored severity of organ dysfunction (by Brussels score) by calculating days alive and free  
10 (DAF) of organ dysfunction (respiratory, acute lung injury, cardiovascular, vasopressors, renal, coagulation, International Normalized Ratio for Partial Thromboplastin Time (INR), hepatic, and neurological (CNS) as well as systemic inflammatory response syndrome (SIRS with all 4 of 4 criteria (SIRS 4 of 4))) over 28 days. PHASE and MEGA 2 were used to determine the haplotypes of protein C in Caucasians. We then genotyped  
15 haplotype tag SNP's that tagged each of the major haplotype clades of each patient.

Patients were well matched by genotype and haplotype at baseline. We found that there were 3 major haplotype clades of protein C (xx, yy, zz %).

20 A novel clade was tagged by protein C T 4732 C and was associated with decreased 28-day survival (54 %, 60 % vs. 68 %, 4732 CC, CT, and TT respectively,  $p < 0.05$  by Fisher's Exact Test) and with increased severity (measured as fewer DAF) of vasopressor use, renal, coagulation (platelets), INR, and hepatic dysfunction (all preceding have  $p < 0.05$ ) as well as more severe renal dysfunction (Spearman's rho) (See Table 8 below).

25

**Table 8A. Days alive and free (DAF) of vasopressors, coagulation (platelets), INR, renal, hepatic SIRS 4 of 4 and neurological (CNS) dysfunction in critically ill patients who had Systemic Inflammatory Response Syndrome (SIRS)**

Genotype of Protein C 4732	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal
PC 4732 TT	18.8±11.1	19.8±11.1	19.1±11.3	17.8±11.6
PC 4732 CT	16.9±11.4	18.5±11.2	18±11.4	15.7±12.2
PC 4732 CC	15.6±11.2	16.9±10.6	16.8±10.9	15.8±10.5
P value	<0.05	<0.06	<0.05	<0.10
	Mean±Standard	Mean±Standard	Mean±Standard	Mean±Standard

	Deviation	Deviation	Deviation	Deviation
Genotype of Protein C 4732	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	
PC 4732 TT	20.1±11	19.5±10.4	19.3±11	
PC 4732 CT	18.8±11.2	18±10.7	17.9±11.4	
PC 4732 CC	15.5±12	16.2±10.3	16.1±11.7	
p	<0.06	<0.05	<0.11	
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	

The association of protein C 4732 C with decreased 28 day survival (57 % vs. 68%, protein C 4732 CC vs. protein C 4732 CT,TT,  $p < 0.05$  by Kaplan Meier) and increased organ dysfunction (use of vasopressors, coagulation (platelets), INR, renal, hepatic SIRS 4 of 4, neurological (CNS) dysfunction and use of inotropic agents (inotropes) was especially pronounced in patients ( $n= 395$  Caucasians) who had sepsis (See Table 8B below).

10 **Table 8B. Days alive and free (DAF) of vasopressors, coagulation (platelets), INR, renal, hepatic dysfunction, SIRS 4 of 4 criteria, neurological (CNS) dysfunction and use of inotropic agents (Inotropes) in critically ill patients who had Sepsis**

Genotype of Protein C 4732	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal
PC 4732 TT	18.6±10.9	20.2±10.7	19.3±11.1	18±11.3
PC 4732 CT	16±11.3	17.8±11.3	17.3±11.4	14.9±12
PC 4732 CC	15.9±10.6	17.2±10.1	17±10.4	15.8±9.9
P value	<0.01	<0.01	<0.012	<0.02
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
PC 4732 TT	20.4±10.7	19.4±10.1	19.4±10.5	20.7±10.3
PC 4732 CT	18±11.1	17.2±10.6	17±11.4	18.6±11.3
PC 4732 CC	15.4±11.9	16.4±9.6	16.3±11.3	19.5±10.3
P value	<0.008	<0.01	<0.06	<0.05

	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	
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A novel clade of protein C tagged by protein C 4732 C is a useful predictor decreased survival and increased multiple organ dysfunctions in SIRS and in sepsis.

- 5 In a subsequent analysis of an increased patient group, 690 Caucasians patients admitted to our ICU with sepsis and enrolled in this study, 518 were successfully genotyped for the protein C 4732 T/C polymorphism and were included in this analysis. The frequency of the alleles is shown in Table 9A. These alleles were in Hardy-Weinberg equilibrium in our population. There were no significant differences in baseline characteristics of
- 10 patients who had the C allele (CC or CT genotype) and those who did not (TT genotype) (Table 9A). Patients were of similar age, gender distribution, had similar APACHE II scores on admission to the ICU and were admitted with similar ratios of medical/surgical diagnoses.
- 15 For the data shown in Tables 9A and 9B below inclusion criteria for patients was as follows. All patients admitted to the ICU of St. Paul's Hospital were screened for inclusion. The ICU is a mixed medical-surgical ICU in a tertiary care, university-affiliated teaching hospital. Patients were included in the study if they met at least two out of four SIRS criteria: 1) fever ( $> 38^{\circ}\text{C}$ ) or hypothermia ( $< 36^{\circ}\text{C}$ ), 2) tachycardia ( $> 90$
- 20 beats/minute), 3) tachypnea ( $> 20$  breaths/minute),  $\text{PaCO}_2 < 32$  mm Hg, or need for mechanical ventilation, and 4) leukocytosis (total leukocyte count  $> 12,000 \text{ mm}^3$ ) or leukopenia ( $< 4,000 \text{ mm}^3$ ). Patients were included in the cohort on the calendar day on which the SIRS criteria were met. Patient who had sepsis were defined by having at least
- 25 2 of 4 SIRS criteria plus suspected or documented infection plus one new organ dysfunction believed to be secondary to sepsis (according to definitions of the Society of Critical Care Medicine). Patients were excluded if blood could not be obtained for genotype analysis. Otherwise the procedures for determining clinical phenotype, haplotype, genotypes and data analysis were as described above.
- 30 Table 9A shows the genotype frequencies of T4732C and baseline characteristics of critically ill patients who had sepsis by genotype of the protein C 4732 T/C

polymorphism.. These alleles were in Hardy Weinberg equilibrium in our population.

There were no significant differences in baseline characteristics of patients who had the C allele (CC or CT genotype) and those who did not (TT genotype). Patients were of similar age, gender distribution, had similar APACHE II scores on admission to the ICU and were admitted with similar ratios of medical/surgical diagnoses.

**TABLE 9A**

<b>Baseline Characteristic</b>	<b>4732 TT</b>	<b>4732 CT</b>	<b>4732 CC</b>	<b>p Value</b>
<b>N (%)</b>	289 (56%)	199 (38%)	30 (6%)	0.099*
<b>Age (Years)</b>	58 ± 1	57 ± 1	58 ± 3	0.742
<b>Gender (% Male)</b>	65	67	70	0.826
<b>APACHE II Score</b>	23 ± 1	25 ± 1	24 ± 1	0.127
<b>Medical/Surgical Diagnosis (% Surgical)</b>	25	25	33	0.596

\*Exact test for Hardy-Weinberg equilibrium of Guo and Thompson.

Patients who had the C allele of the protein C 4732 T/C polymorphism had significantly lower 28-day survival, as indicated by a two-way Fisher exact test (Table 9A) or a Kaplan-Meier 28-day survival curve (Figure 7). Patients with the C allele of the protein C 4732 TC polymorphism (CC or CT genotype) had a 28-day survival rate of 58%, while those without the C allele (TT genotype) had a 28-day survival rate of 67% (p=0.035).

Table 9B shows Survival and days alive and free (DAF) of organ dysfunction by protein C 4732 T/C genotype in critically ill patients with sepsis.

**TABLE 9B.**

	<b>4732 CC/CT</b>	<b>4732 TT</b>	<b>p Value</b>
<b>Survival</b>	<b>58%</b>	<b>67%</b>	<b>0.035</b>
<b>DAF</b>	<b>16 ± 1</b>	<b>18 ± 1</b>	<b>0.011*</b>
<b>Vasopressors</b>			
<b>DAF Inotropes</b>	<b>19 ± 1</b>	<b>21 ± 1</b>	<b>0.040</b>
<b>DAF 3/4 SIRS</b>	<b>12 ± 1</b>	<b>14 ± 1</b>	<b>0.034</b>
<b>DAF 4/4 SIRS</b>	<b>17 ± 1</b>	<b>19 ± 1</b>	<b>0.011</b>
<b>DAF</b>	<b>18 ± 1</b>	<b>20 ± 1</b>	<b>0.009</b>
<b>Coagulation</b>			
<b>DAF INR &gt; 1.5</b>	<b>17 ± 1</b>	<b>19 ± 1</b>	<b>0.014</b>
<b>DAF</b>	<b>11 ± 1</b>	<b>13 ± 1</b>	<b>0.088</b>
<b>Ventilation</b>			
<b>DAF Renal Dysfunction</b>	<b>15 ± 1</b>	<b>17 ± 1</b>	<b>0.060</b>
<b>DAF CNS Dysfunction</b>	<b>17 ± 1</b>	<b>19 ± 1</b>	<b>0.092</b>
<b>DAF Hepatic Dysfunction</b>	<b>17 ± 1</b>	<b>20 ± 1</b>	<b>0.006</b>

\*Significance tested by Spearman's Rho correlation.

- 5 Patients with the CC or CT genotype of the protein C 4732 polymorphism had significantly greater vasopressor and inotrope use, as indicated by significantly fewer days alive and free of vasopressor and inotrope use. Patients with the CC or CT genotype of the protein C 4732 polymorphism had significantly greater inflammation as indicated by significantly fewer days alive and free of 3/4 and 4/4 SIRS criteria. Patients with the CC
- 10 or CT genotype of the protein C 4732 T/C polymorphism had significantly greater coagulation as indicated by significantly fewer days alive and free of coagulation and days alive and free of INR > 1.5. Patients with the C allele of the protein C 4732 T/C polymorphism had trends toward greater ventilation and greater renal dysfunction as indicated by fewer days alive and free of mechanical ventilation and renal dysfunction.
- 15 Patients who had the CC or CT genotype of the protein C 4732 T/C polymorphism had a trend toward greater neurological dysfunction and had significantly greater hepatic dysfunction as indicated by fewer days alive and free of CNS and hepatic dysfunction.

### EXAMPLE 3: Combination of EPCR and Protein C Polymorphisms

An interaction of novel haplotypes of protein C (protein C 4732 C) and endothelial protein C receptor (EPCR 4054 T) is associated with decreased survival and increased organ dysfunction in sirs, sepsis and septic shock

Patients who had no copies of the EPCR risk allele (4054T) and no copies of the protein C risk allele (4732C) had the best 28 day survival and the least severity of organ dysfunction (protective-protective). Furthermore, patients who had at least one copy of the EPCR risk allele (4054T) and at least one copy of the protein C risk allele (4732C) had the lowest survival and the greatest organ dysfunction (risk-risk). Finally, patients who had either no copies of the EPCR risk allele (4054T) and at least one copy of the protein C risk allele (4732C) or who had at least one copy of the EPCR risk allele (4054T) and no copies of the protein C risk allele (4732C) had intermediate survival and organ dysfunction. These findings are interesting and suggest that the interaction of SNP haplotypes of protein C and EPCR are important predictors of the outcomes of critically ill patients who have SIRS.

Our results cannot be explained by differences in the baseline characteristics of the patients classified into our groups 1, 2 and 3 as there were no differences in important predictors of outcome including age, APACHE II score, proportion of patients who had sepsis at onset of the study and proportion of patients who had septic shock at the onset of the study.

Previously it was not known whether interactions of risk alleles of protein C and risk alleles of EPCR were associated with altered outcomes in systemic inflammatory response syndrome (SIRS) or sepsis. We show that interactions of alleles of protein C and EPCR that are associated with increased risk of poor outcome ("risk alleles") is associated with increased risk of death and organ dysfunction in systemic inflammatory response syndrome (SIRS), sepsis and septic shock.

Our study was based on an inception cohort of 487 critically ill Caucasian patients who met at least 2/4 SIRS criteria. We defined subgroups who had sepsis (n= 393) and who had septic shock (n = 260). Outcomes were 28-day survival and severity of organ dysfunction by calculating days alive and free (DAF) of organ dysfunction (Brussels score: respiratory, cardiovascular, renal, coagulation, International Normalized Ratio for Partial Thromboplastin Time (INR) < 1.5, hepatic, and neurological dysfunction and use of vasopressors, inotropic agents, and renal support by continuous renal replacement therapy or dialysis (renal support)). Haplotypes and clades of protein C and EPCR were determined by PHASE and MEGA 2 in Caucasians. We selected haplotype tag SNP's that tagged each haplotype clade. We previously found novel haplotypes with risk alleles of protein C (tagged by 4732 C) and EPCR (4054 T) associated with increased risk of death and organ dysfunction. Therefore, we classified patients into 3 groups as having copies of protein C and EPCR risk alleles defined as follows:

**Risk – Risk Group 1:** defined patients who had at least 1 copy of the risk allele of protein C 4732 C and at least 1 copy of the EPCR 4054 T.

**Risk – Protective Group 2:** defined patients who had no risk alleles of protein C 4732 C and at last 1 copy of EPCR 4054 T OR at least 1 copy of the protein C 4732 C and no copies of the EPCR 4054 T.

**Protective – Protective Group 3:** defined patients who had no copies of the protein C 4732 C and no copies of the EPCR 4054 T (wild type).

<u>EPCR</u>	<u>SNP 4054</u>	<u>Designation</u>
	4054T	Risk
	4054C	Protective

<u>ProC</u>	<u>SNP 4732</u>	<u>Designation</u>
	4732C	Risk
	4732T	Protective

We then tested for associations of these 3 risk groups (Risk-Risk; Risk-Protective; Protective-Protective) with 28 day survival and with organ dysfunction as scored by days alive and free of organ dysfunction.

Patients with SIRS in the Protective-Protective Group had 28 day survival of 73.7 %, patients in the Risk-Protective Group had 28 day survival of 67 %, and patients in the Risk-Risk Group had 28 day survival of 58.4 % ( $p < 0.02$  by Chi-square;  $p < 0.03$  by Kaplan-Meier survival analysis over 28 days).

The organ dysfunction of patients who had SIRS according to group is shown in Table 10. There was a steady increase in organ dysfunction (scored as lower days alive and free of organ dysfunction and support) from Protective-Protective, through Risk-Protective to Risk-Risk groups.

5

**Table 10. Days alive and free (DAF) of use of vasopressors, coagulation (platelets) dysfunction, INR, renal, cardiovascular dysfunction, hepatic dysfunction, SIRS 4 of 4, neurological (CNS) dysfunction and use of inotropic agents (inotropes) in 487 critically ill patients who had Systemic Inflammatory Response Syndrome (SIRS) according to group**

10

PC 4732 C/EPCR 4054T Risk Group	DAF Vasopressor s	DAF Coagulation	DAF INR	DAF Renal	DAF CVS
Risk-Risk	16.1±11.3	18±11.2	17.6±11.5	15.2±12	14.5±11.2
Risk- Protective	18.5±11.3	19.5±11.3	19±11.4	17.6±11.6	16.1±11.2
Protective - Protective	20.4±10.4	21.5±10	20±10.5	19.5±11.2	18.8±10.6
P value	<0.003	<0.06	<0.05	<0.10	<0.018
	Mean±Stand ard Deviation	Mean±Stand ard Deviation	Mean±Stand ard Deviation	Mean±Stand ard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
Risk-Risk	18.1±11.3	17.3±10.6	17.2±11.5	18.8±11.3

Risk-Protective	19.8±11.1	19.2±10.5	19±11	20.2±10.7
Protective-Protective	20.8±11.1	21±10	21±10.4	22.1±9.8
P value	<0.06	<0.004	<0.11	<0.034
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

Significance for days alive and free of organ dysfunction tested by Spearman's rho statistic.

- 5 Patients with sepsis (n=393) in the Protective-Protective Group had 28 day survival of 70.3 %, patients in the Risk-Protective Group had 28 day survival of 67 %, and patients in the Risk-Risk Group had 28 day survival of 56 % (p< 0.04 by Kaplan-Meier survival analysis over 28 days).
- 10 The organ dysfunction of patients who had sepsis according to group is shown in Table 11. There was a steady increase in organ dysfunction (scored as lower days alive and free of organ dysfunction and support) from Protective-Protective, through Risk-Protective to Risk-Risk groups.
- 15 **Table 11. Days alive and free (DAF) of use of vasopressors, coagulation (platelets) dysfunction, INR, renal, cardiovascular dysfunction, hepatic dysfunction, SIRS 4 of 4, neurological (CNS) dysfunction, use of inotropic agents (inotropes), and renal support in 393 critically ill patients who had Sepsis according to group**

PC 4732 C/EPCR 4054T Risk Group	DAF Vasopressors	DAF Coagulation	DAF INR	DAF Renal	DAF CVS
Risk-Risk	15.6±11.1	17.7±11.1	17.2±11.4	14.7±11.8	13.4±11
Risk-	18.4±11.1	19.8±10.9	19±11.2	17.6±11.4	15.8±11

Protective					
Protective-Protective	19.3±10.4	21.2±10	19.7±10.2	19.4±11	17.6±10.5
P value	<0.007	<0.031	<0.036	<0.006	<0.055
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Hepatic	DAF SIRS 4 of 4	DAF CNS	DAF Inotropes
Risk-Risk	17.7±11.2	16.9±10.4	16.6±11.4	18.5±11.2
Risk-Protective	19.9±10.9	19.1±10.2	19.1±10.7	20.4±10.5
Protective-Protective	20.8±11	20.1±10	20.3±10.3	21.8±10
P value	<0.028	<0.007	<0.021	<0.013
	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation	Mean±Standard Deviation

PC 4732 C/EPCR 4054T Risk Group	DAF Renal Support
Risk-Risk	16.1±11.9
Risk-Protective	18.1±11.8
Protective-Protective	17.8±12.2
P value	< 0.09
	Mean±Standard Deviation

- 5 Significance for days alive and free of organ dysfunction tested by Spearman's rho statistic.

Patients with septic shock (n = 260) in the Protective-Protective Group had 28 day survival of 63 %, patients in the Risk-Protective Group had 28 day survival of 60 %, and

patients in the Risk-Risk Group had 28 day survival of 50 % ( $p < 0.107$  by Kaplan-Meier survival analysis over 28 days).

We conclude that there is an interaction between risk alleles of protein C (4732C) and EPCR (4054T) (as defined above) that is associated with increased risks of death and multiple organ dysfunctions in systemic inflammatory response syndrome (SIRS), sepsis and septic shock.

**EXAMPLE 4: Improved Response to Therapy with Activated Protein C (XIGRIS™)**

Therapies for sepsis may include mechanical ventilation, support of circulation with vasopressors and inotropic agents, antibiotics, drainage of abscesses and surgery as appropriate. Activated protein C (APC or XIGRIS™ (when referring to APC as sold by Eli Lilly & Co., Indianapolis IN)) can improve survival of sepsis patients. The PROWESS trial showed that XIGRIS™ decreased 28 day mortality from 31 % (placebo) to 25 % (APC/XIGRIS™ – treated). XIGRIS™ was particularly effective in patients at high risk of death for example as identified by having an APACHE II score greater than or equal to 25. XIGRIS™ has been approved for treatment of severe sepsis at increased risk of death. In some jurisdictions, the high risk of death is identified as having an APACHE II score greater than or equal to 25; in other jurisdictions high risk of death is identified as having 2 or more organ dysfunctions or having an APACHE II score greater than or equal to 25.

All genotyping, clinical phenotyping, sample collection, sample analysis and statistical methods were performed as described herein. Patients with clinically defined sepsis, treated with activated protein C (XIGRIS™) and who had been genotyped for the protein C and EPCR polymorphisms were the subject of Example 4. A total of 893 patients were studied. Baseline characteristics and 28 day survival of each group (treated with XIGRIS™ and not treated with XIGRIS™) were determined and 38 patients were compared who had sepsis and were treated with XIGRIS™ to patients who had sepsis, but were not treated with XIGRIS™.

The severity of organ dysfunction that occurs in the setting of sepsis was also examined to determine whether XIGRIS™ reduces organ dysfunction in patients who have sepsis and

who have an at risk genotype of protein C or EPCR (protein C 2418 A, protein C 4732 C and EPCR 4054 T).

To determine the severity of organ dysfunction the Brussels scoring system was used.

- 5 Each patient was assessed for each organ dysfunction on each day over 28 days after meeting at least two of four criteria for the systemic inflammatory response syndrome (SIRS). On each day, each organ system dysfunction was classified in each patient as being present (if organ dysfunction was moderate, severe or extreme according to the Brussels score) or absent (normal or mild according to the Brussels score). Days alive and
- 10 free of organ dysfunction were calculated each day (from day 0 to day 28). Each day a patient had an organ dysfunction or was dead was scored as a zero for that organ dysfunction. In contrast, each day that a person was both alive and free of an organ dysfunction was scored as a one for that organ dysfunction. Then, the scores for each organ dysfunction of each patient were summed over the 28-day observation period.
- 15 Thus, a score could range from 0 to 28. A higher score is beneficial because it indicates more days alive and free of organ dysfunction.

#### **EXAMPLE 4A: Baseline Characteristics**

- The baseline characteristics of patients who had severe sepsis and who were treated with
- 20 XIGRIS™ are shown in Table 12. These patients are typical of patients who have severe sepsis in that the average age was 54 years, the average APACHE II score was 31, there was frequent organ dysfunction and the survival was 58 percent.

- 25 **TABLE 12. Baseline characteristics, survival and organ dysfunction (as days alive and free of organ dysfunction) of 38 patients who had sepsis and were treated with activated protein C (XIGRIS™).**

	MEAN
AGE	54.1
APACHE II	31
SURGICAL (%)	26
SURVIVAL (% 28 DAY)	58
DAF ALI	12.1
DAF PRESSORS	15.1
DAF INOTROPES	17.5
DAF SIRS 4/4	15.4
ADMISSION SEPSIS (%)	100

ADMISSION SEPTIC SHOCK	83
(%)	
DAF STEROIDS	11.2
DAF CVS	13.2
DAF RESP	11
DAF PF 300	4
DAF VENTILATION	10.3
DAF CNS	15.8
DAF COAGULATION	15.9
DAF INR > 1.5	17.3
DAF RENAL	14.3
DAF RENAL SUPPORT	14.1
DAF HEPATIC	15.7

#### EXAMPLE 4B: Protein C 4732 Genotype

The effects of treatment with XIGRIST<sup>TM</sup> according to the genotype of patients for protein

5 C 4732 T/C is shown in Table 13 below.

**Table 13. Outcomes of patients who were treated with activated protein C (XIGRIST<sup>TM</sup>) according to protein C 4732 genotype TT vs. CT, CC).**

Protein C 4732 TT (non-risk genotype)	MEAN (n=18)	Protein C 4732 CT and CC (risk genotype)	MEAN (n=10)
AGE	49.4	AGE	56.5
APACHE II	30	APACHE II	32
SURGICAL (%)	28	SURGICAL (%)	10
SURVIVAL (% 28 DAY)	61	SURVIVAL (% 28 DAY)	70 %
DAF ALI	11	DAF ALI	11.1
DAF PRESSORS	16.6	DAF PRESSORS	17.1
DAF INOTROPES	18.3	DAF INOTROPES	18.8
DAF SIRS 4/4	16.6	DAF SIRS 4/4	15.6
ADMISSION SEPSIS (%)	100	ADMISSION SEPSIS (%)	100%
ADMISSION SEPTIC SHOCK (%)	87	ADMISSION SEPTIC SHOCK (%)	86 %
DAF STEROIDS	13.4	DAF STEROIDS	12.6
DAF CVS	14.7	DAF CVS	14.9
DAF RESP	12.1	DAF RESP	11.7
DAF PF > 300	5.9	DAF PF > 300	2.9
DAF VENTILATION	11.3	DAF VENTILATION	11.2
DAF CNS	17.7	DAF CNS	14.5
DAF COAGULATION	17.7	DAF COAGULATION	16.8
DAF INR > 1.5	18.9	DAF INR > 1.5	20
DAF RENAL	15.9	DAF RENAL	16
DAF RENAL SUPPORT	15.3	DAF RENAL SUPPORT	12.1
DAF HEPATIC	17.6	DAF HEPATIC	16.9

Patients who had protein C 4732 CC and CT and were treated with XIGRIST<sup>TM</sup> had somewhat greater age and APACHE II score, than patients who were genotype protein C 4732 TT, yet the 4732 CC and CT patients had higher survival (70 percent compared to 61 percent) than patients who were genotype 4732 TT and treated with XIGRIST<sup>TM</sup>.

Survival of patients who had sepsis and were not treated with XIGRIST<sup>TM</sup> were compared to patients who had sepsis and were treated with XIGRIST<sup>TM</sup> according to protein C 4732 genotype as shown in Table 14 below.

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**Table 14. Survival of patients who had severe sepsis who were treated/not treated with XIGRIST<sup>TM</sup> according to their genotype of protein C 4732.**

Protein C 4732	Sepsis not treated with XIGRIST <sup>TM</sup> Survival (%)	Sepsis and treated with XIGRIST <sup>TM</sup> – Survival (%)	APACHE II of XIGRIST <sup>TM</sup> -treated
4732 TT	68	61 N = 18	30
4732 CC, CT	57	70 N = 10	32

15 Patients who had the risk genotype (protein C 4732 CC, CT) and were not treated with XIGRIST<sup>TM</sup> had a survival of 57 percent, whereas treatment with XIGRIST<sup>TM</sup> increased survival of patients who were protein C 4732 CC, CT to 70 %. Thus, XIGRIST<sup>TM</sup> increased the absolute survival rate by 13 percent (70 percent – 57 percent = 13 percent) of patients who had the at risk genotype of protein C 4732.

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Another way to examine the effect of XIGRIST<sup>TM</sup> on survival is to evaluate the relative increase in survival. XIGRIST<sup>TM</sup> increased the survival of patients who had severe sepsis who had the at risk genotype of protein C 4732 CC by 23 percent relatively ( $13/57 \times 100$ ) compared to protein C 4732 CT and TT. Another useful comparison to evaluate the effects of XIGRIST<sup>TM</sup> on survival according to protein C 4732 genotype is to compare the survival of protein C 4732 TT (wild type) in patients not treated with XIGRIST<sup>TM</sup> to patients who were genotype of protein C 4732 TT (wild type) and were treated with XIGRIST<sup>TM</sup>. Of the patients who were not treated with XIGRIST<sup>TM</sup>, the survival of wild type genotype of protein C 4732 (i.e. protein C 4732 TT) was higher than the risk genotype (protein C 4732 CC, CT) - 68 percent survival vs. 57 percent survival (wild type not treated with XIGRIST<sup>TM</sup>). In contrast, the treatment with XIGRIST<sup>TM</sup> did not improve the survival of patients who had the wild type (non-risk or protective) genotype (protein C

30

4732 TT) (61 percent survival if treated with XIGRIS™ vs. 68 percent survival if not treated with XIGRIS™). The treatment with XIGRIS™ increased the survival of patients who had the risk genotype (protein C 4732 CC, CT) such that their survival (70 percent) was higher than the survival of patients who had the wild type genotype of protein C 4732 who were treated with XIGRIS™ (70 percent vs. 67 percent).

#### EXAMPLE 4C: EPCR 4054 Genotype

As shown in Table 15, there was a slight increase in survival of patients who had the genotype EPCR 4054 TT who were treated with XIGRIS™ (70 percent survival) compared to patients who had the EPCR 4054 TT genotype who were not treated with XIGRIS™ (survival = 63 percent).

**Table 15. Comparison of survival of patients with sepsis who were not treated with XIGRIS™ to patients with sepsis who were treated with XIGRIS™ according to EPCR 4054 genotype.**

EPCR 4054	Sepsis survival not treated with XIGRIS™ (%)	Sepsis survival – treated with XIGRIS™ (%)	APACHE II
CC/CT	66	40 N = 5	37
TT	63	70 N = 23	29

#### EXAMPLE 4D: Combined Genotype of Protein C 4732 and EPCR 4054

XIGRIS™ had the greatest beneficial increase in survival in patients who had the combined risk – risk genotypes of protein C 4732 and EPCR 4054 (Table 16).

**Table 16. Comparison of survival of patients with sepsis who were not treated with XIGRIS™ to patients with sepsis who were treated with XIGRIS™ according to protein C/EPCR genotype (protective – protective (non-risk – non-risk), risk – protective (risk – non-risk) pooled as “all others”, risk – risk).**

Protein C 4732 – EPCR 4054	Sepsis survival (%)	APC – treated survival (%)	APACHE II
All others	68	62 N = 21	30
Risk – Risk	58	83 N = 6	30

XIGRIS™ treatment increased survival of risk – risk compared to patients not treated with APC (58 % to 83 %), an absolute increase in survival of 25 percent. Another way to evaluate the effects of XIGRIS™ is to examine the relative increase in survival. XIGRIS™ increased the relative chance of survival of the risk – risk genotype of protein C 4732 and EPCR 4054 by 43 percent ( $25/58 \times 100$ ).

Of the patients who had sepsis and received XIGRIS™ the protective – protective and risk – protective (wherein protective = non-risk) genotypes of protein C 4732 and EPCR 4054 were pooled because of the small number of patients in the protective – protective group.

The survival of the pooled protective – protective and risk – protective groups who received XIGRIS™ was 62 percent; in contrast, the survival of the patients who had sepsis who had the risk – risk combined genotype who received XIGRIS™ was higher at 83 percent. Thus, in another useful comparison, XIGRIS™ treatment increased the survival of sepsis patients having the risk – risk genotype of protein C 4732 and EPCR 4054, such that it exceeded the survival of the pooled protective – protective and risk – protective groups who received XIGRIS™ by 21 percent (62 percent vs. 83 percent). XIGRIS™ increased the survival of patients who had the risk – risk combined genotype of protein C 4732 C and EPCR 4054 T.

XIGRIS™ also increased survival of patients who had the risk – protective haplotypes compared to risk – protective patients who were not treated with XIGRIS™ from 67 to 71 %, an absolute increase in survival of 4 percent, and a relative increase in survival of 6 percent.

#### **EXAMPLE 4E: SUMMARY OF EFFECTS OF XIGRIS™ ON PROTEIN C 2418, PROTEIN C 4732, EPCR 4054 AND THE COMBINED PROTEIN C 4732/EPCR 4054 GENOTYPES**

Table 17 summarizes the effects of XIGRIS™ on survival of patients according to the protein C 2418, protein C 4732, EPCR 4054 and the combined protein C 4732/EPCR 4054 genotypes. The control group of ‘Sepsis survival – Not treated with Xigris™’ includes all evaluable patients, many of whom were not eligible for Xigris treatment and had an APACHE II score measurably lower than treated patients.

**Table 17. Summary of effects of XIGRIS™ on protein C 2418, protein C 4732, EPCR 4054 and the combined protein C 4732/EPCR 4054 genotypes.**

Genotype	Sepsis survival – Not treated with XIGRIS™	Sepsis survival – Treated with XIGRIS™	Absolute Increase in Survival with XIGRIS™	Relative Increase in Survival with XIGRIS™
EPCR 4054 TT	63 %	70 %	7 %	10%
Protein C 2418 AA	58 %	64 %	6 %	10 %
Protein C 4732 CC/CT	57 %	70 %	13 %	23 %
Protein C 4732 CC/ EPCR 4054 TT (Risk – Risk)	58 %	83 %	25 %	43 %

Even with a biased control sample, there was an internally consistent graded absolute increase in survival prediction according to the EPCR 4054, protein C 2418, protein C 4732, 4054 and the combined protein C 4732/EPCR 4054 genotypes and a useful prediction of benefit from treatment with XIGRIS™.

XIGRIS™ provided a graded increase in survival according to genotype. For example, XIGRIS™ provided an absolute increase in survival of 7 percent for the EPCR 4054 TT, 6 percent for the protein C 2418 AA, 13 percent for the protein C 4732 CC, CT, and 25 percent for the protein C 4732 CC, CT and EPCR 4054 TT combined risk – risk genotype.

#### **EXAMPLE 4E.1: SUMMARY OF EFFECTS OF XIGRIS™ ON PROTEIN C 2418, PROTEIN C 4732 AND THE COMBINED PROTEIN C 4732/EPCR 4054 GENOTYPES**

Table 18 summarizes the effects of XIGRIS™ treatment on survival of patients by genotype compared to a control group of untreated patients having APACHE II scores of 25 or higher (n=302). This control group compares more closely to the XIGRIS™ treatment group than the control group in Table 17 because an APACHE II score of 25 or greater is a minimum requirement for approved use of XIGRIS™.

**Table 18 Summary of effects of XIGRIS™ on protein C 2418, protein C 4732 and the combined protein C 4732/EPCR 4054 genotypes.**

Genotype	Sepsis Survival APACHE II $\geq$ 25 not treated with Xigris™	Sepsis survival – Treated with XIGRIS™	Absolute Increase in Survival with XIGRIS™	Relative Increase in Survival with XIGRIS™
Protein C 2418 AA	46%	68%	22%	48%
Protein C 4732 CC/CT	41%	70%	29%	70%
Protein C 4732 CC/ EPCR 4054 TT (Risk – Risk)	44%	83%	39%	89%

There was a statistically significant absolute increase in survival with XIGRIS™ in patients with a risk genotype than would be expected from a randomized patient population. (Generally, about 13% absolute increase in survival is expected in randomized patients with sepsis. (PROWESS trial)). A patient carrying such a polymorphism is expected to respond much better than patients with a decreased risk genotype.

**EXAMPLE 4F: Effects of Treatment with XIGRIS™ on Organ Dysfunction According to Protein C 4732 Genotype**

Table 19 shows that patients who had the risk – risk genotype of Protein C 4732 C/EPCR 4054 T who were treated with XIGRIS™ had less organ dysfunction as shown by more days alive and free of organ dysfunction and organ support than did patients who had the protein C 4732 C/EPR 4054 T risk – risk genotype who had sepsis and who were not treated with XIGRIS™. In particular, patients treated with XIGRIS™ had less need for vasopressors (more DAF Pressors), less need for inotropes (more DAF inotropes), less cardiovascular dysfunction (more DAF Cardiovascular), less respiratory dysfunction (more DAF Respiratory), less need for ventilation (more DAF Ventilation), less coagulopathy (more DAF INR > 1.5), and less hepatic dysfunction (more DAF hepatic).

**Table 19. Comparison of organ dysfunction scored as days alive and free (DAF) of organ dysfunction and organ support of patients who had sepsis who were not treated with XIGRIS™ to patients who had sepsis who were treated with XIGRIS™**

according to protein C 4732 C genotype/EPCR 4054 T genotype in the risk – risk subgroup (as defined below in detailed patent) .

Protein C 4732 C – EPCR 4054 T Risk - Risk	Sepsis not treated with XIGRIS™	XIGRIS™-treated N = 6
Age	58	56
APACHE II	25	30
DAF Pressors	15.6	19.5
DAF Inotropes	18.5	21.8
DAF Cardiovascular	13.9	16.5
DAF Respiratory	11.4	15
DAF Ventilation	10.4	14.3
DAF CNS	16.6	16.5
DAF INR > 1.5	17.2	23.7
DAF Renal	14.7	14
DAF Hepatic	17.7	18.5

- 5 The above results demonstrate that risk genotypes of the protein C and/or EPCR genes are associated with significantly greater response to therapy or improved response. Patients who present with an inflammatory condition such as SIRS, and who carry a risk genotype, are likely to benefit from treatment significantly more than persons with non-risk or protective genotypes.

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#### Clinical Implications

- Patients with sepsis, severe sepsis or SIRS may be genotyped to assess their protein C 2418, protein C 4732 and EPCR 4054 genotypes or the genotypes of polymorphism sites in linkage disequilibrium with protein C 2418, protein C 4732 and EPCR 4054. Patients could then be classified by genotype into a risk category regarding their unique risk of death by genotype. Furthermore, the patient's genotype can be used to also determine how well they will respond to activated protein C (XIGRIS™) or other anti-inflammatory agents or anti-coagulant agents. It was found that there was a clear graded increase in absolute survival when XIGRIS™ was used as treatment according to the genotype of the patient for EPCR 4054, protein C 2418, protein C 4732, and the combined risk – risk genotype of protein C 4732/EPCR 4054. Thus, clinicians can now administer XIGRIS™ according to a patient-tailored risk assessment. Each patient's unique genotype of protein C and EPCR can be used to make a unique assessment of risk of death and to predict whether or not a patient is likely to benefit from XIGRIS™ treatment.

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Although the foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity of understanding, it will be readily apparent to those of skill in the art in light of the teachings of this invention that changes and modification may be made thereto without departing from the spirit or scope of the appended claims.

- 5 All patents, patent applications and publications referred to herein are hereby incorporated by reference.

## CLAIMS

### What is Claimed is:

1. A method for obtaining a prognosis for a patient having or at risk of developing an inflammatory condition, the method comprising determining a genotype including one or more polymorphism sites in the protein C gene; EPCR gene or a combination thereof for the patient, wherein said genotype is indicative of an ability of the patient to recover from the inflammatory condition.
2. The method of claim 1, wherein a polymorphism site corresponds to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.
3. The method of claim 1, wherein polymorphism sites from both the Protein C gene and EPCR gene are combined, wherein said polymorphism sites correspond to one or more of position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or position 2418 of SEQ ID NO:1; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.
4. The method of claim 2 or 3, wherein the polymorphism site in linkage disequilibrium with position 4732 corresponds to position 4813, 6379, 6762, 7779, 8058, 8915 or 12228 in SEQ ID NO: 1.
5. The method of claim 2 or 3, wherein the polymorphism site in linkage disequilibrium with position 4054 corresponds to position 2973, 3063, 3402, 4946, 5515 or 6196 in SEQ ID NO: 2.
6. The method of claim 3, wherein the polymorphism site in linkage disequilibrium with position 2418 corresponds to position 1386, 2583 or 3920 in SEQ ID NO: 1.
7. The method of claim 2 or 3, wherein the polymorphism site in linkage

disequilibrium with position 4732 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

9198 and 5867;  
9198 and 4800;  
3220 and 5867; and  
3220 and 4800.

8. The method of claim 3, wherein the polymorphism site in linkage disequilibrium with position 2418 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

5867 and 2405;  
5867 and 4919;  
5867 and 4956;  
5867 and 6187;  
5867 and 9534;  
5867 and 12109;  
4800 and 2405;  
4800 and 4919;  
4800 and 4956;  
4800 and 6187;  
4800 and 9534;  
4800 and 12109;  
9198 and 6379 and 2405;  
9198 and 6379 and 4919;  
9198 and 6379 and 4956;  
9198 and 6379 and 6187;  
9198 and 6379 and 9534; and  
9198 and 6379 and 12109.

9. The method of any one of claims 1-8, further comprising comparing the genotype so determined with known genotypes, which are indicative of a prognosis for recovery from the same inflammatory condition as for the patient or another inflammatory condition.

10. The method any one of claims 1-9, further comprising obtaining protein C gene or EPCR gene sequence information for the patient.

11. The method any one of claims 1-9, wherein said determining of genotype is performed on a nucleic acid sample from the patient.

12. The method of claim 11, further comprising obtaining a nucleic acid sample from the patient.

13. The method any one of claims 1-12, wherein said determining of genotype comprises one or more of:

- (a) restriction fragment length analysis;
- (b) sequencing;
- (c) hybridization;
- (d) oligonucleotide ligation assay;
- (e) ligation rolling circle amplification;
- (f) 5' nuclease assay;
- (g) polymerase proofreading methods;
- (h) allele specific PCR; and
- (i) reading sequence data.

14. The method of any one of claims 1-13, wherein the genotype of the patient is indicative of a decreased likelihood of recovery from an inflammatory condition or an increased risk of having a poor outcome.

15. The method of claim 14, wherein the prognosis is indicative of severe cardiovascular or respiratory dysfunction in critically ill patients.

16. The method of claim 14 or 15, wherein the genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 C;  
4813 A;  
6379 G;  
6762 A;  
7779 C;  
8058 T;  
8915 T;

5                   12228 T;  
                   9198 C and 5867 A;  
                   9198 C and 4800 G;  
                   3220 A and 5867 A; and  
                   3220 A and 4800 G  
  
                   or  
  
 10                  1386 T;  
                   2418 A;  
                   2583 A;  
                   3920 T;  
                   5867 A and 2405 T;  
                   5867 A and 4919 A;  
 15                  5867 A and 4956 T;  
                   5867 A and 6187 C;  
                   5867 A and 9534 T;  
                   5867 A and 12109 T;  
 20                  4800 G and 2405 T;  
                   4800 G and 4919 A;  
                   4800 G and 4956 T;  
                   4800 G and 6187 C;  
                   4800 G and 9534 T;  
                   4800 G and 12109 T;  
 25                  9198 A and 6379 G and 2405 T;  
                   9198 A and 6379 G and 4919 A;  
                   9198 A and 6379 G and 4956 T;  
                   9198 A and 6379 G and 6187 C;  
                   9198 A and 6379 G and 9534 T; and  
 30                  9198 A and 6379 G and 12109 T.

17. The method of claim 14 or 15, wherein the genotype is selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

35                  6196 G;  
                   5515 T;  
                   4946 T;  
                   4054 T;  
                   3402 G;  
                   3063 G; and  
 40                  2973 C.

18. The method of any one of claims 1-13, wherein the genotype of the patient is indicative of an increased likelihood of recovery from an inflammatory condition.

19. The method of claim 18, wherein the prognosis is indicative of less severe cardiovascular or respiratory dysfunction in critically ill patients.

20. The method of claim 18 or 19, wherein the genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 T;  
4813 G;  
6379 A;  
6762 G;  
7779 -;  
8058 C;  
8915 G;  
12228 C;  
9198 A and 5867 G;  
9198 A and 4800 C;  
3220 G and 5867 G; and  
3220 G and 4800 C

or

1386 C;  
2418 G;  
2583 T;  
3920 C;  
5867 G and 2405 C;  
5867 G and 4919 G;  
5867 G and 4956 C;  
5867 G and 6187 T;  
5867 G and 9534 C;  
5867 G and 12109 C;  
4800 C and 2405 C;  
4800 C and 4919 G;  
4800 C and 4956 C;  
4800 C and 6187 T;  
4800 C and 9534 C; and  
4800 C and 12109 C.

21. The method of claim 18 or 19, wherein the genotype is selected from the group of EPCR single polymorphism sites in SEQ ID NO: 2 consisting of:

6196 C;  
5515 C;  
4946 C;  
4054 C;

3402 C;  
3063 A; and  
2973 T.

- 5     22.     The method of any one of claims 1-21, wherein the inflammatory condition is  
selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock,  
systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress  
Syndrome (ARDS), acute lung injury, aspiration pneumonia, infection,  
pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to  
10     trauma, inflammation due to surgery, chronic inflammatory disease, ischemia,  
ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease,  
tissue damage due to chemotherapy or radiotherapy, and reactions to ingested,  
inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel  
infection, opportunistic infections, and for patients undergoing major surgery or  
15     dialysis, patients who are immunocompromised, patients on immunosuppressive  
agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with  
fever, patients with fever of unknown origin, patients with cystic fibrosis, patients  
with diabetes mellitus, patients with chronic renal failure, patients with  
bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis,  
20     emphysema, or asthma, patients with febrile neutropenia, patients with meningitis,  
patients with septic arthritis, patients with urinary tract infection, patients with  
necrotizing fasciitis, patients with other suspected Group A streptococcus  
infection, patients who have had a splenectomy, patients with recurrent or  
suspected enterococcus infection, other medical and surgical conditions associated  
25     with increased risk of infection, Gram positive sepsis, Gram negative sepsis,  
culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome,  
cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure,  
hepatitis, epiglottitis, E. coli 0157:H7, malaria, gas gangrene, toxic shock  
syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis,  
30     Pneumocystis carinii, pneumonia, Leishmaniasis, hemolytic uremic  
syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever,  
pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr  
virus, encephalitis, inflammatory diseases and autoimmunity including

Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

23. The method of any one of claims 1-22, wherein the inflammatory condition is systemic inflammatory response syndrome.

24. A method of identifying a polymorphism in a protein C gene sequence that correlates with a patient prognosis, the method comprising:

- a) obtaining protein C gene or EPCR sequence information from a group of patients;
- b) identifying a site of at least one polymorphism in the protein C or EPCR gene;
- c) determining genotypes at the site for individual patients in the group;
- d) determining an ability of individual patients in the group to recover from the inflammatory condition; and
- e) correlating genotypes determined at step (c) with patient abilities determined at step (d).

25. The method of claim 24, wherein the inflammatory condition is selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumonia, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel infection, opportunistic infections, and for patients undergoing major surgery or dialysis,

patients who are immunocompromised, patients on immunosuppressive agents,  
 patients with HIV/AIDS, patients with suspected endocarditis, patients with fever,  
 patients with fever of unknown origin, patients with cystic fibrosis, patients with  
 diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis,  
 patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or  
 asthma, patients with febrile neutropenia, patients with meningitis, patients with  
 septic arthritis, patients with urinary tract infection, patients with necrotizing  
 fasciitis, patients with other suspected Group A streptococcus infection, patients  
 who have had a splenectomy, patients with recurrent or suspected enterococcus  
 infection, other medical and surgical conditions associated with increased risk of  
 infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis,  
 fungal sepsis, meningococemia, post-pump syndrome, cardiac stun syndrome,  
 myocardial infarction, stroke, congestive heart failure, hepatitis, epiglottitis, E. coli  
 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia,  
 HELLP Syndrome, mycobacterial tuberculosis, Pneumocystis carinii, pneumonia,  
 Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura,  
 Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme  
 disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and  
 autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic  
 sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic  
 pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis,  
 Wegener's granulomatosis, transplants including heart, liver, lung kidney bone  
 marrow, graft-versus-host disease, transplant rejection, sickle cell anemia,  
 nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and  
 cirrhosis.

26. A kit for determining a genotype at a defined nucleotide position within a polymorphism site in a protein C gene or an EPCR gene from a patient to provide a prognosis of the patient's ability to recover from an inflammatory condition, the kit comprising, a restriction enzyme capable of distinguishing alternate nucleotides at the polymorphism site or a labeled oligonucleotide having sufficient complementary to the polymorphism site and capable of distinguishing said alternate nucleotides.

27. The kit of claim 26, wherein a polymorphism site corresponds to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.
28. The kit of claim 26, wherein the kit is suitable for determining genotype at one or more nucleotide positions within each of the protein C gene or the EPCR gene, wherein said polymorphism sites correspond to one or more of position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or position 2418 of SEQ ID NO:1; or a polymorphism site in linkage disequilibrium with position 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.
29. The kit of claim 26, 27 or 28 further comprising an oligonucleotide or a set of oligonucleotides suitable to amplify a region including the polymorphism site.
30. The kit of claim 29, further comprising a polymerization agent.
31. The kit of any one of claims 26-30, further comprising instructions for using the kit to determine genotype.
32. A method for selecting a group of patients for determining the efficacy of a candidate drug known or suspected of being useful for the treatment of an inflammatory condition, the method comprising determining a genotype for one or more polymorphism sites in the protein C gene or EPCR gene for each patient, wherein said genotype is indicative of the patient's ability to recover from the inflammatory condition and sorting patients based on their genotype.
33. The method of claim 32 further comprising, administering the candidate drug to the patients or a subset of patients and determining each patient's ability to recover from the inflammatory condition.
34. The method of claim 33, further comprising comparing patient response to the

candidate drug based on genotype of the patient.

35. A method of treating an inflammatory condition in a patient in need thereof, the method comprising administering to the patient an anti-inflammatory agent or an anti-coagulant agent, wherein said patient has a protein C gene or EPCR gene risk genotype.

36. A method of treating an inflammatory condition in a patient in need thereof, the method comprising:

- (a) selecting a patient having a risk genotype in their protein C gene or EPCR gene; and
- (b) administering to said patient an anti-inflammatory agent or an anti-coagulant agent.

37. A method of treating an inflammatory condition in a mammal in need thereof, the method comprising administering to the mammal an anti-inflammatory agent or an anti-coagulant agent, wherein said mammal has a protein C gene or EPCR gene risk genotype.

38. A method of treating an inflammatory condition in a mammal, the method comprising:

- (a) selecting a mammal having a risk genotype in their protein C gene or EPCR gene; and
- (b) administering an anti-inflammatory agent or an anti-coagulant agent to the selected mammal.

39. A method of selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of identifying a subject having one or more risk genotypes in their protein C gene or EPCR gene, wherein the identification of a subject with one or more risk genotypes is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.

40. A method of treating a subject with an inflammatory condition by administering an anti-inflammatory agent or an anti-coagulant agent, the method comprising administering the anti-inflammatory agent or the anti-coagulant agent to subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.
41. A method of identifying a subject with increased responsiveness to treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of screening a population of subjects to identify those subjects that have a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with a risk genotype in their protein C gene or EPCR gene is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.
42. A method of selecting a subject for the treatment of an inflammatory condition with an anti-inflammatory agent or an anti-coagulant agent, comprising the step of identifying a subject having a risk genotype in their protein C gene or EPCR gene, wherein the identification of a subject with the risk genotype is predictive of increased responsiveness to the treatment of the inflammatory condition with the anti-inflammatory agent or the anti-coagulant agent.
43. A method of treating an inflammatory condition in a patient, the method comprising administering an anti-inflammatory agent or an anti-coagulant agent to the patient, wherein said patient has a risk genotype in their protein C gene or EPCR gene.
44. A method of treating an inflammatory condition in a patient, the method comprising:
- (a) identifying a patient having a risk genotype in their protein C gene or EPCR gene; and

(b) administering an anti-inflammatory agent or an anti-coagulant agent to the patient.

- 5 45. The use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament for the treatment of an inflammatory condition, wherein the patients treated have a risk genotype in their protein C gene or EPCR gene.
- 10 46. The use of an anti-inflammatory agent or an anti-coagulant in the manufacture of a medicament for the treatment of an inflammatory condition in a subset of patients, wherein the subset of patients have a risk genotype in their protein C gene or EPCR gene.
- 15 47. The method of any one of claims 35 to 44, further comprising determining the patients APACHE II score as an assessment of patient risk.
48. The method of any one of claims 35 to 44 and 47, further comprising determining the number of organ system failures for the patient as an assessment of patient risk.
- 20 49. The method of claim 48, wherein the patients APACHE II score is indicative of increased risk when  $\geq 25$ .
50. The method of claim 49, wherein 2 or more organ system failures are indicative of increased patient risk.
- 25 51. The method of any one of claims 35 to 50, wherein the inflammatory condition is selected from the group consisting of: sepsis, septicemia, pneumonia, septic shock, systemic inflammatory response syndrome (SIRS), Acute Respiratory Distress Syndrome (ARDS), acute lung injury, aspiration pneumonitis, infection, pancreatitis, bacteremia, peritonitis, abdominal abscess, inflammation due to
- 30 trauma, inflammation due to surgery, chronic inflammatory disease, ischemia, ischemia-reperfusion injury of an organ or tissue, tissue damage due to disease, tissue damage due to chemotherapy or radiotherapy, and reactions to ingested, inhaled, infused, injected, or delivered substances, glomerulonephritis, bowel

infection, opportunistic infections, and for patients undergoing major surgery or dialysis, patients who are immunocompromised, patients on immunosuppressive agents, patients with HIV/AIDS, patients with suspected endocarditis, patients with fever, patients with fever of unknown origin, patients with cystic fibrosis, patients with diabetes mellitus, patients with chronic renal failure, patients with bronchiectasis, patients with chronic obstructive lung disease, chronic bronchitis, emphysema, or asthma, patients with febrile neutropenia, patients with meningitis, patients with septic arthritis, patients with urinary tract infection, patients with necrotizing fasciitis, patients with other suspected Group A streptococcus infection, patients who have had a splenectomy, patients with recurrent or suspected enterococcus infection, other medical and surgical conditions associated with increased risk of infection, Gram positive sepsis, Gram negative sepsis, culture negative sepsis, fungal sepsis, meningococcemia, post-pump syndrome, cardiac stun syndrome, myocardial infarction, stroke, congestive heart failure, hepatitis, epiglottitis, E. coli 0157:H7, malaria, gas gangrene, toxic shock syndrome, pre-eclampsia, eclampsia, HELP syndrome, mycobacterial tuberculosis, Pneumocystis carinii, pneumonia, Leishmaniasis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura, Dengue hemorrhagic fever, pelvic inflammatory disease, Legionella, Lyme disease, Influenza A, Epstein-Barr virus, encephalitis, inflammatory diseases and autoimmunity including Rheumatoid arthritis, osteoarthritis, progressive systemic sclerosis, systemic lupus erythematosus, inflammatory bowel disease, idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, systemic vasculitis, Wegener's granulomatosis, transplants including heart, liver, lung kidney bone marrow, graft-versus-host disease, transplant rejection, sickle cell anemia, nephrotic syndrome, toxicity of agents such as OKT3, cytokine therapy, and cirrhosis.

52. The method of any one of claims 35-51, wherein the inflammatory condition is systemic inflammatory response syndrome.

53. The method of any one of claims 35-52, wherein the risk genotype is located at a polymorphism site corresponding to position 4732 of SEQ ID NO:1; or position 4054 of SEQ ID NO:2; or a polymorphism site in linkage disequilibrium with 4732

of SEQ ID NO:1 or position 4054 of SEQ ID NO:2.

54. The method of any one of claims 35-52, wherein the risk genotype is located at a polymorphism site corresponding to position 2418 of SEQ ID NO:1 or a polymorphism site in linkage disequilibrium thereto.

55. The method of any one of claims 35-52, wherein the risk genotypes from the Protein C gene and EPCR gene are located at polymorphism sites corresponding to one or more of position 4732 of SEQ ID NO:1; position 4054 of SEQ ID NO:2; position 2418 of SEQ ID NO:1; and a polymorphism site in linkage disequilibrium with 4732 of SEQ ID NO:1 or position 4054 of SEQ ID NO:2 or position 2418 of SEQ ID NO:1.

56. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4732 corresponds to position 4813, 6379, 6762, 7779, 8058, 8915 or 12228 in SEQ ID NO: 1.

57. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4054 corresponds to position 2973, 3063, 3402, 4946, 5515 or 6196 in SEQ ID NO: 2.

58. The method of claim 54 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 2418 corresponds to position 1386, 2583 or 3920 in SEQ ID NO: 1.

59. The method of claim 53 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 4732 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

9198 and 5867;  
9198 and 4800;  
3220 and 5867; and  
3220 and 4800.

60. The method of claim 54 or 55, wherein the risk genotype located at a polymorphism site in linkage disequilibrium with position 2418 corresponds to a combination of two or more Protein C polymorphism sites, the combination being selected from the group of positions in SEQ ID NO: 1 consisting of:

5867 and 2405;  
5867 and 4919;  
5867 and 4956;  
5867 and 6187;  
5867 and 9534;  
5867 and 12109;  
4800 and 2405;  
4800 and 4919;  
4800 and 4956;  
4800 and 6187;  
4800 and 9534;  
4800 and 12109;  
9198 and 6379 and 2405;  
9198 and 6379 and 4919;  
9198 and 6379 and 4956;  
9198 and 6379 and 6187;  
9198 and 6379 and 9534; and  
9198 and 6379 and 12109.

61. The method of any one of claims 53, 54, 55, 56, 58, 59 or 60, wherein the genotype for an increased risk or risk genotype is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 C;  
4813 A;  
6379 G;  
6762 A;  
7779 C;  
8058 T;  
8915 T;  
12228 T;  
9198 C and 5867 A;  
9198 C and 4800 G;  
3220 A and 5867 A; and  
3220 A and 4800 G

or

5                   1386 T;  
                   2418 A;  
                   2583 A;  
                   3920 T;  
                   5867 A and 2405 T;  
                   5867 A and 4919 A;  
                   5867 A and 4956 T;  
                   5867 A and 6187 C;  
 10                  5867 A and 9534 T;  
                   5867 A and 12109 T;  
                   4800 G and 2405 T;  
                   4800 G and 4919 A;  
                   4800 G and 4956 T;  
 15                  4800 G and 6187 C;  
                   4800 G and 9534 T;  
                   4800 G and 12109 T;  
                   9198 A and 6379 G and 2405 T;  
                   9198 A and 6379 G and 4919 A;  
 20                  9198 A and 6379 G and 4956 T;  
                   9198 A and 6379 G and 6187 C;  
                   9198 A and 6379 G and 9534 T; and  
                   9198 A and 6379 G and 12109 T.

25   62.   The method of any one of claims 53, 55 or 57, wherein the genotype for an  
           increased risk or risk genotype is selected from the group of EPCR single  
           polymorphism sites and combined polymorphism sites in SEQ ID NO: 2 consisting  
           of:

30                   6196 G;  
                   5515 T;  
                   4946 T;  
                   4054 T;  
                   3402 G;  
                   3063 G; and  
 35                   2973 C.

63.   The method of any one of claims 61 or 62, wherein the genotype of the patient is  
        indicative of an increased risk of poor outcome from an inflammatory condition.

40   64.   The method of any one of claims 61, 62 or 63, wherein the patient having an  
           increased risk of poor outcome from an inflammatory condition is preferentially

selected for administration the anti-inflammatory agent or the anti-coagulant agent.

65. The method of any one of claims 53, 54, 55, 56, 58, 59 or 60, wherein the genotype for a decreased risk is selected from the group of protein C single polymorphism sites and combined polymorphism sites in SEQ ID NO: 1 consisting of:

4732 T;  
4813 G;  
6379 A;  
6762 G;  
7779 -;  
8058 C;  
8915 G;  
12228 C;  
9198 A and 5867 G;  
9198 A and 4800 C;  
3220 G and 5867 G; and  
3220 G and 4800 C

or

1386 C;  
2418 G;  
2583 T;  
3920 C;  
5867 G and 2405 C;  
5867 G and 4919 G;  
5867 G and 4956 C;  
5867 G and 6187 T;  
5867 G and 9534 C;  
5867 G and 12109 C;  
4800 C and 2405 C;  
4800 C and 4919 G;  
4800 C and 4956 C;  
4800 C and 6187 T;  
4800 C and 9534 C; and  
4800 C and 12109 C.

66. The method of any one of claims 53, 55 or 57, wherein the genotype for a decreased risk is selected from the group of EPCR single polymorphism sites and combined polymorphism sites in SEQ ID NO: 2 consisting of:

6196 C;  
5515 C;  
4946 C;  
4054 C;

3402 C;  
3063 A; and  
2973 T.

- 5     67.     The method of any one of claims 65 or 66, wherein the genotype of the patient is indicative of a decreased risk of poor outcome from an inflammatory condition.
68.     The method of any one of claims 65, 66 or 67, wherein the patient having a decreased risk of poor outcome from an inflammatory condition is preferentially  
10     not selected for administration the anti-inflammatory agent or the anti-coagulant agent.
69.     The method of any one claims 35 to 67, wherein the anti-inflammatory agent or the anti-coagulant agent is selected from any one or more of the following:
- 15             (a)     activated protein C;  
              (b)     tissue factor pathway inhibitors;  
              (c)     platelet activating factor hydrolase;  
              (d)     PAF-AH enzyme analogues;  
              (e)     antibody to tumor necrosis factor alpha;  
20             (f)     soluble tumor necrosis factor receptor-immunoglobulin G1;  
              (g)     procysteine;  
              (h)     elastase inhibitor;  
              (i)     human recombinant interleukin 1 receptor antagonists; and  
              (j)     antibodies, inhibitors and antagonists to endotoxin, tumour necrosis  
25             factor receptor, interleukin-6, high mobility group box, tissue plasminogen activator, bradykinin, CD-14 and interleukin-10.
70.     The method of any one claims 35 to 69, wherein the anti-inflammatory agent or the anti-coagulant agent is activated protein C.
- 30     71.     The method of any one claims 35 to 70, wherein the anti-inflammatory agent or the anti-coagulant agent is drotrecogin alfa activated.

### **ABSTRACT**

The invention provides methods and kits for obtaining a prognosis for a patient having or at risk of developing an inflammatory condition and for identifying patients having a greater benefit from treatment with an anti-inflammatory agent or an anti-coagulant agent.

- 5 The method generally comprises determining a protein C and/or EPCR genotype(s) of a patient for a polymorphisms in the these genes, comparing the determined genotype with known genotypes for the polymorphism that correspond with the ability of the patient to recover from the inflammatory condition and identifying patients based on their prognosis.

- 10 The invention also provides for methods of identifying potential patients having an inflammatory condition who are more likely to benefit from treatment with an anti-inflammatory agent or anti-coagulant agent and subsequent to treatment recover from the inflammatory condition. The invention also provides for methods of treating such patients with an anti-inflammatory agent or anti-coagulant agent based on the patient's genotype.

## FIGURE 1

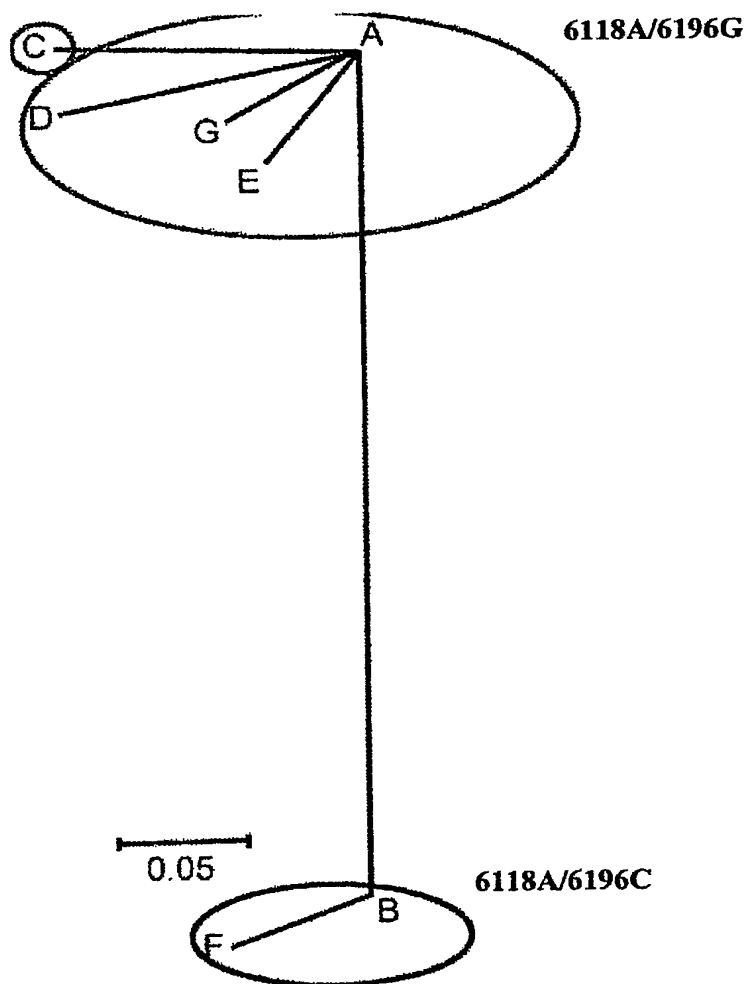
[illegible]

FIGURE 2

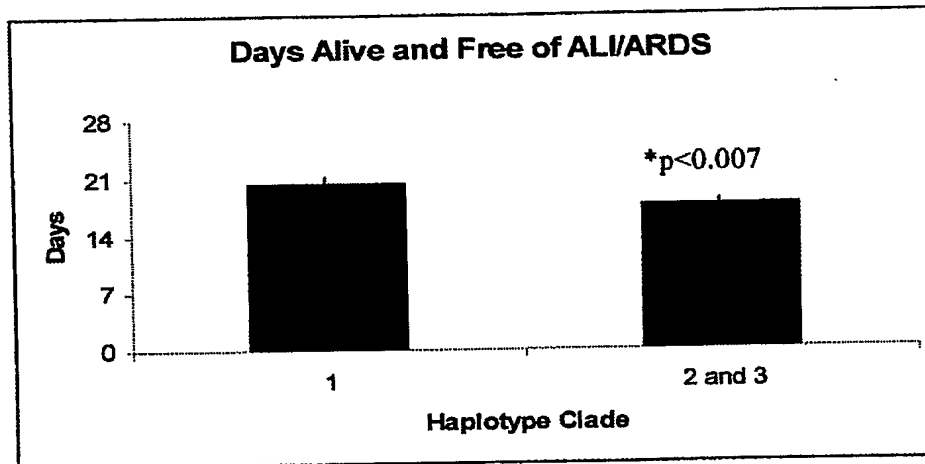
Position															
837	1940	2313	2973	3063	3402	3514	3600	4054	4946	5329	5515	6118	6196		
C	G	A	C	G	G	G	T	T	T	G	T	A	G		
C	A	A	C	G	G	G	T	T	T	G	T	A	G		
C	G	A	C	G	G	A	T	T	T	G	T	A	G		
C	G	A	C	G	G	G	C	T	T	A	T	A	G		
G	G	A	C	G	G	G	T	T	T	G	T	G	G		
C	G	A	T	A	C	G	T	C	C	G	C	A	C		
C	G	C	T	A	C	G	T	C	C	G	C	A	C		

**FIGURE 3**

6118G/6196G



**FIGURE 4**



**FIGURE 5**

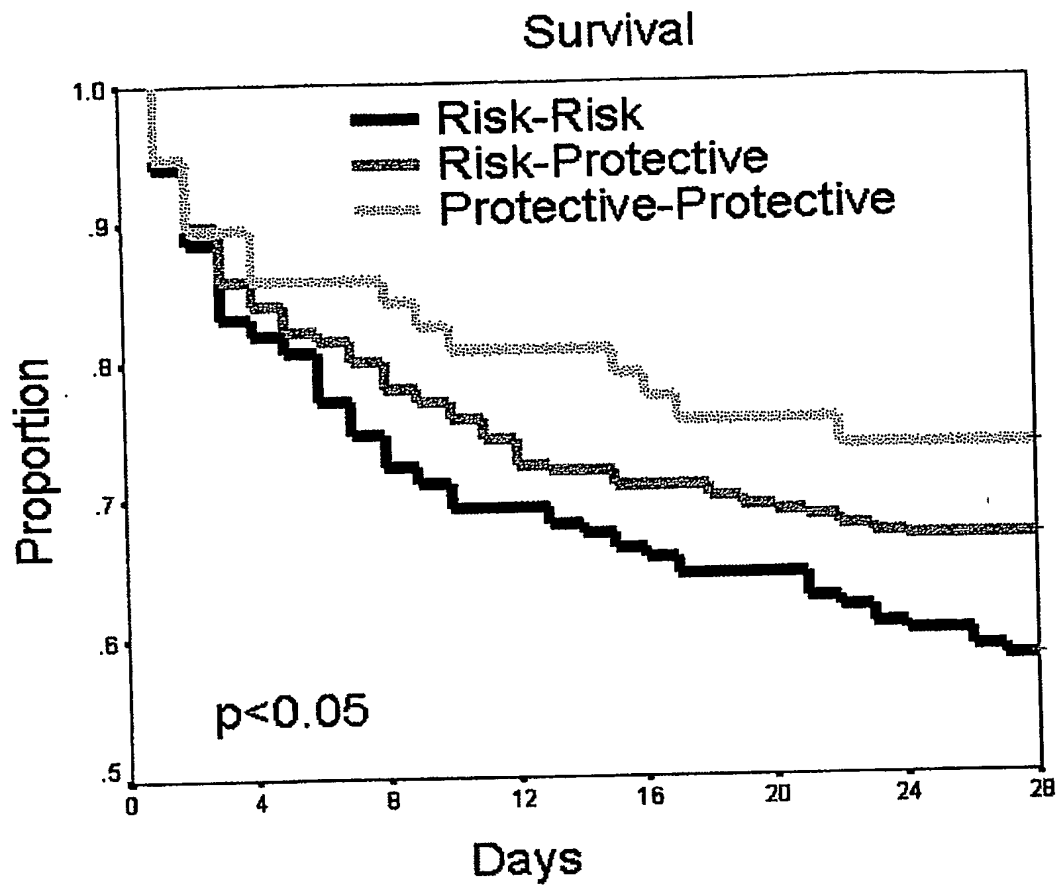
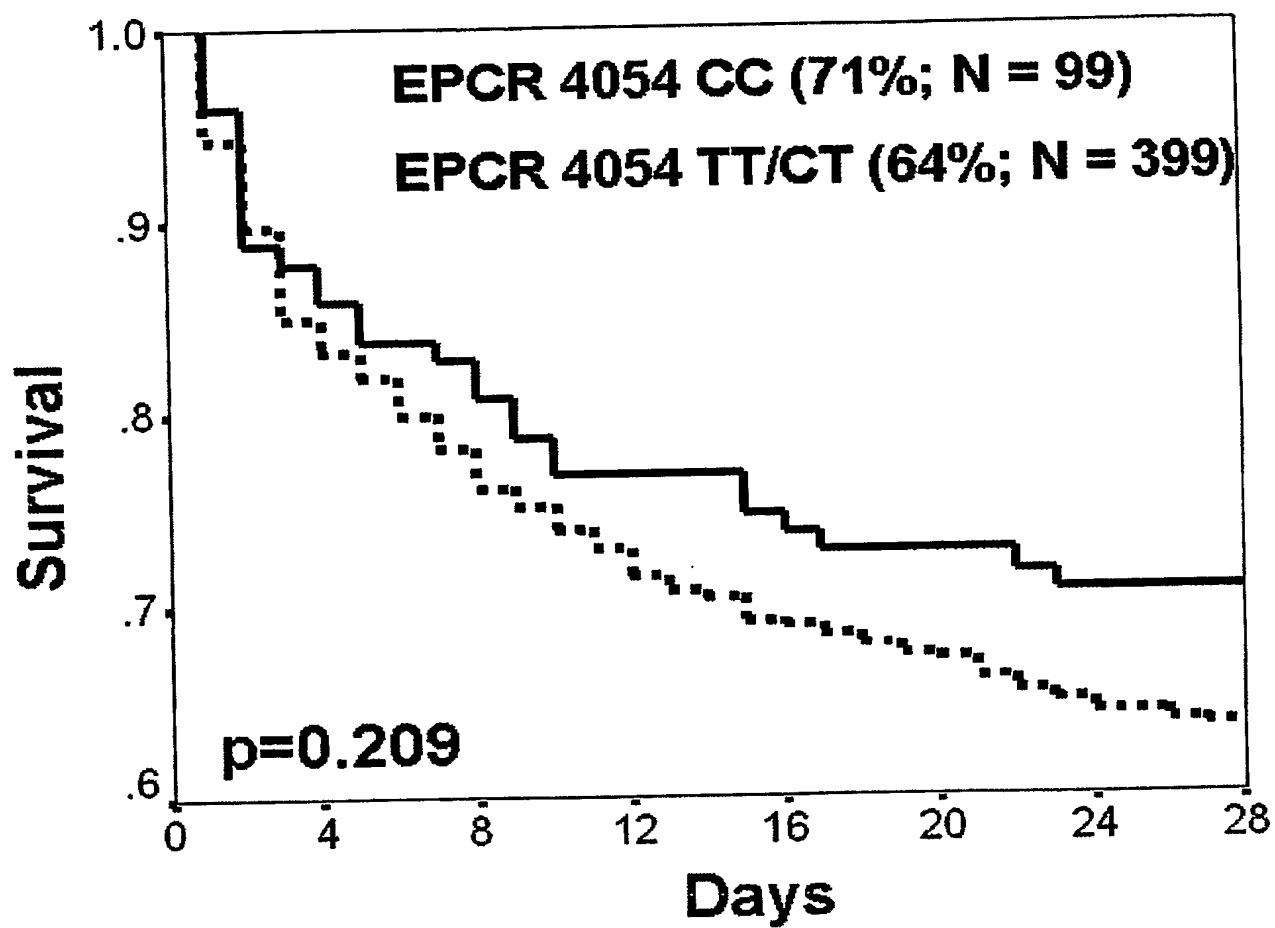
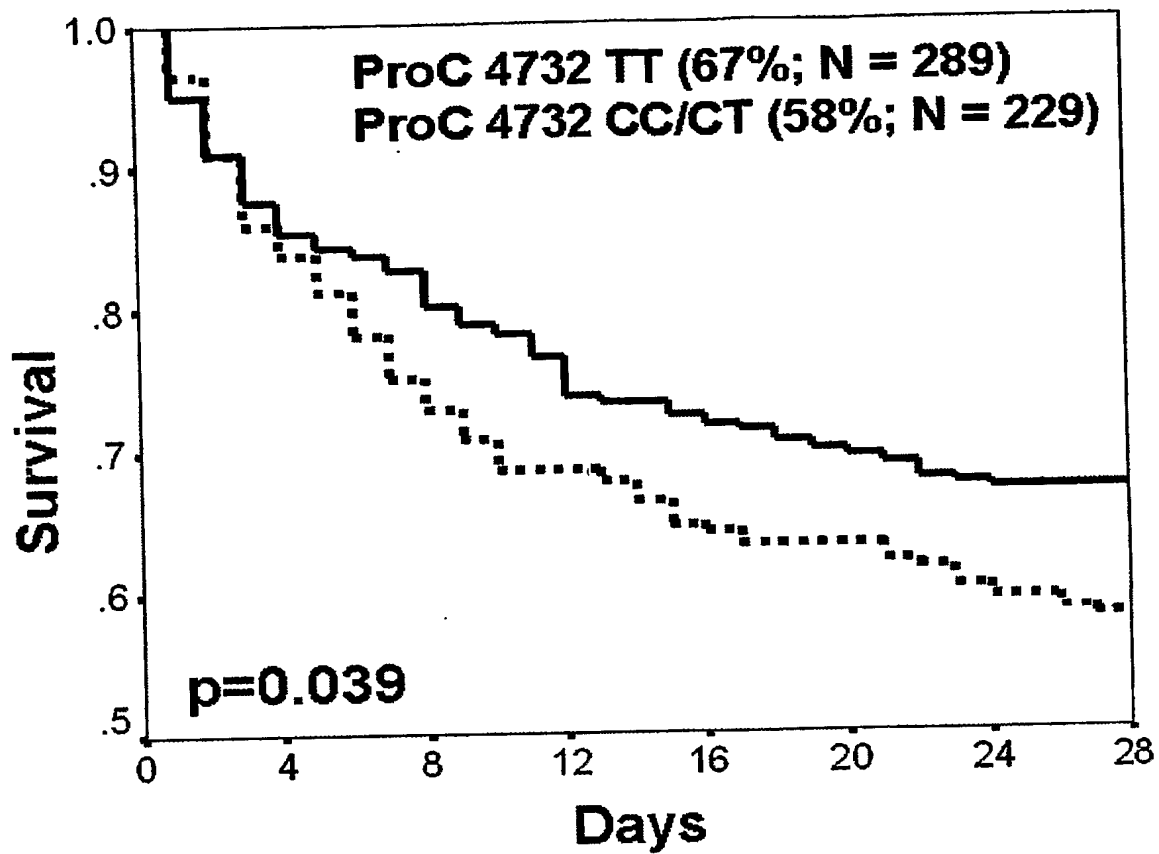


FIGURE 6



**FIGURE 7**



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